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Joseph C. Gilman, President, 1952

MYCOLOGIA

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THE PURE CULTURE IN TAXONOMY[1]

JOSEPH C. GILMAN[2]

(WITH PORTRAIT)

In 1881 Lankester, commenting on the announcement of Koch at the International Medical Congress in London that year, of his use of the gelatin plate for culturing bacteria, made the following statement:

"It is only by such monosporous cultivations that we can arrive at solid conclusions in reference to the forms and activities of the bacteria, e.g. as to whether one form can give rise to progeny of another form when its food and conditions of growth are changed, and again, as to whether special fermentative powers can be lost or acquired in the course of generations derived from one parent germ, but subjected to different conditions as to food, temperature and oxygen."

While this statement was concerned with the bacteria its implications in respect to fungi are obvious.

From the standpoint of techniques, Koch's use of gelatin culture was the turning point in laboratory practice in both bacteriology and mycology. It is true that pure cultures had been obtained earlier by dilution methods as devised by Pasteur and Lister. These

[1] Journal paper No. J-2163 of the Agricultural Experiment Station, Ames, Iowa. Project No. 1018.

[2] Presidential address, Mycological Society of America. Presented September 8, 1952, at the Society meetings in Ithaca, N. Y.

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methods were cumbersome and laborious and the results therefrom were uncertain. When the investigator could combine the dilution series with subsequent growth on a solid medium, with the single cells fixed in space on the surface, his results were clear and he could proceed to further experiments with confidence.

Culturing of micro-organisms, fungi and bacteria was not new. Mycologists like Brefeld and DeBary had cultured their fungi many years before, but they worked largely in the same manner as a gardener, growing their organisms in spite of contamination. Pasteur and Lister were using solid media—such materials as potato plugs that were opaque and not amenable to the critical examination made possible by the transparent background of the gelatin film.

Soon Frau Hesse, working in Koch's laboratory, announced the use of agar-agar as the base for culture-media and the foundations for modern culture techniques were laid. From then on, this tool was available and the work of adapting it to its many modern uses and keeping it sharp for critical study has been the labor of mycologists, bacteriologists and biochemists. It is my purpose to examine the record of the seventy years that have passed and discuss some of the advances made possible by the use of this tool. How far have Lankester's predictions been confirmed?

His "solid conclusions" were to be found in reference to the "forms" of the organisms and to the "activities" of them. In other words, how far have we advanced in our knowledge of morphology of the fungi and with it of the taxonomy that is based on such morphology and what advances have been made in our knowledge of fungous physiology? Let us examine these two fields separately, discussing the latter first.

In the field of physiology there have been tremendous advances, particularly in regard to the substrate, and from that in regard to the nutritional needs of many fungi. We might divide our discussion into three parts: mineral nutrition, vitamins and antibiotics.

By means of the pure culture the effects of minerals, vitamins, sugars and proteins on the growth and sporulation of fungi have been extensively and intensely studied. Steinberg, for example, has shown that amounts of particular mineral elements far below the level measurable by the ordinary chemical balance affect the

growth rate, coloration and sporulation of fungi to a degree that is readily recognizable by gross examination. Similar studies on the vitamin requirements of fungi in culture have been demonstrated by Robbins and Lilly and their associates. On the other hand Raper and Whiffen have demonstrated the extreme variability present in pure cultures when single spore isolates from a single colony of a fungus were plated and the progeny grown to maturity. A somewhat similar variation, in virulence rather than morphologic characteristics, was encountered when strains of *Phytophthora infestans* from potato and tomato were grown from single zoospores and the progeny compared with regard to their pathogenicity. Examples of this kind might be multiplied indefinitely. Mass transfers, in contrast, tend to maintain a uniform type of colony. Such reactions are most readily explained by postulating a wide variability in the genetic composition of the mycelial nuclei in any particular colony of the fungus. In these studies the uniformity of substratum on which the progeny was grown was unquestionably accepted in the first case or, in the case of pathogenicity studies, distinctions, such as tomato plants and potato plants, were clearly defined. However, when fungi are isolated and studied in different laboratories or even at different times in the same laboratory, this variability might well account for multiplicity of species from either single spore cultures or the selectivity of the medium used for a particular study.

Granted that great genetic variability is present in most fungous thalli, if we accept the inadequacies of the chemical balance to measure biological quantities of reagents and the lack of biological purity in most of the commercial supplies of the commonly used chemicals as well as chemical contaminants in any but especially prepared glassware, should we not expect biological variants to appear regularly in our cultures?

To cloud the issue further, the needs of fungi for growth and sporulation are varied with respect to vitamin requirements and hormonal action. Not only do these requirements differ from species to species but many of them vary genetically for strains, clones, or individual thalli within a species. Hence these needs must be supplied either by supplementing the medium with one or more constituents or the medium itself should contain them, and

the investigator is forced to use natural substrata, cooked stems, leaves or other plant or animal parts for the growth of his fungi. It might be argued that these natural substrata, if composed of similar parts of similar plants, for example, are more biologically constant than two samples of any synthetic medium. Whether this be the case or not, such natural substrata have been found in practice to be the most available means of meeting the needs of the investigator who required an abundant supply of spores or an ascus stage to carry out his study. In their studies on *Fusarium*, Appel and Wollenweber recommended cooked stems for the production of their "normal" conidia, to name one of the earlier instances of a swing from synthetic to natural media.

From pure culture studies we have a great mass of information concerning the nutritional needs of individual species. As yet no general pattern of behavior is apparent either in respect to physical and chemical responses to environment, or to taxonomic units. Such a situation is probably to be expected with a group as large and diverse as the fungi, yet we are continually trying to find some further orderliness in our taxonomic structure.

Pure cultures have provided an expanding field of research in relation to the production of antibiotics by fungi but with the excellent synopsis by Raper last year, this phase needs no discussion at this time.

Taxonomically the pure culture has provided the basis for great advances. Of primary importance was the connecting of different stages in the cycles of the fungi, making it possible to demonstrate on an experimental basis the connections that had been postulated previously from association and sequence of appearance so ably observed by the Tulasne brothers. With the pure culture came the knowledge of heterothallism, that needs only mention, since it has been ably discussed so recently by Whitehouse. In plant pathology it led to Koch's postulates and to a clearer understanding of parasitism; with the latter came a re-evaluation of the taxonomy of the Imperfecti. Cross-inoculations have shown that many species that were earlier set up on a host basis are not confined to a narrow range of host plants; much synonymy has resulted. This situation unfortunately does not simplify the problem, for many of

the species have common hosts and inoculation trials need be carried out to separate the species involved.

From the point of view of taxonomy the use of pure cultures has given rise to two schools of thought. While I am not sufficiently optimistic to think that anything I may say will bring in an unanimous opinion reconciling these points of view, I wish to examine them somewhat critically as a means of clarifying our thinking. On the one hand we find those investigators who emphasize the fungus and look for a medium for culturing it that will give optimum conditions for its growth and sporulation. Such a medium under proper temperature, moisture, or other environmental conditions should supply the necessary vitamins, minerals, proteins and carbohydrates for completion of the cycle in culture. No universal single medium would serve since we know that the requirements of a given species differ markedly from those of many other species, and also vary with the age of the culture, the length of time the fungus has been separated from its natural substrata, and the degree of specialization toward its substrata that the fungus has acquired. The gamut of conditions is almost infinite, and the crux of the problem still lies within the judgment of the investigator to determine the evasive ideal of what is optimum, or what is normal. The difficulties of making such a judgment have led to the opposing idea: the selection of a single medium on which the growth of the fungi under investigation would be compared and their responses noted. Here the selection of the proper medium becomes paramount. Certain workers have advocated a purely synthetic medium such as Czapek's or a modification of it. Unfortunately such media are definitely limited in their usefulness when the range of cultivable fungi is taken into account. The saprobic molds can be treated in this manner very successfully but the facultative parasites, many of which are serious plant pathogens, do not lend themselves to such treatment, principally because many of them are unable to synthesize the growth substances necessary for fruition or for growth itself. Further criticism of such a procedure might be raised; that in the light of Steinberg's findings, we do not have the implementation necessary to insure sufficiently accurate measurements of ingredients to be confident that any two batches of medium are biologically identical. The sensitivity of the fungus is so great

that it defeats the validity of any comparisons based upon a single medium. Another difficulty in the use of synthetic media arises from the fact that many of the fungi, particularly acervulate and sporodochial forms, seemingly need a particular substrate structure for the formation of such fruits. The sporodochial structure frequently is difficult to demonstrate on agar cultures.

Faced with the above dilemma, the investigator will still find it necessary to accept the situation and govern his procedures by the fungus involved. The pure culture has proved a potent tool but its use still must be mastered. It has taught us much concerning the physiology and genetics of fungi and has been extremely useful in studies of parasitism and the variability of fungi. Taxonomically it still must be used with care, particularly in regard to the Fungi Imperfecti, a group whose taxonomy was so strikingly commented upon by the late Dr. J. J. Davis, whom many of our older members, I am sure, will remember. His comment was:

"You will find a fungus and determine its characteristics. You turn to the books and decide on its genus. Then you look for the species. And you look, and you look, and after a while you find it! In another genus!"

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COPROGEN, A NEW GROWTH FACTOR PRESENT IN DUNG, REQUIRED BY PILOBOLUS SPECIES

C. W. Hesseltine, A. R. Whitehill, C. Pidacks, Margaret Ten Hagen, N. Bohonos, B. L. Hutchings, and J. H. Williams

(with 8 figures)

There are very few references in the literature (1, 2, 3, 4, 5, 7, 8, 9, 12) dealing with the cultivation in pure culture of species of *Pilobolus*. In these reported cases of pure culture, a dung decoction has always been a necessary constituent of the media used for growth. This fact seemed rather remarkable considering the extensive literature on the morphology, light relationships, spore discharge, etc. of this genus. It seemed of interest to secure an isolate of one of these species of *Pilobolus* and to determine its growth requirements. If an unknown growth factor were present, it would be of considerable interest to isolate this compound for further biological evaluation.

ISOLATION AND MAINTENANCE OF CULTURE

In January, 1950, several collections of fresh horse dung obtained from a pasture on the grounds of Lederle Laboratories at Pearl River, New York, were brought into the laboratory and placed in glass dishes to which a few ml. of tap water had previously been added. The dishes were covered to bring about a saturated atmosphere, and then they were placed in a north window. These gross cultures were allowed to stand for several days at room temperature. In making initial isolations it is imperative that only a small amount of water be added to each dish to keep the surrounding atmosphere saturated. Too much water causes water-soaking of the dung balls, leading to excessive multiplication of bacteria, which is unfavorable for *Pilobolus* growth. On the fourth day a great abundance of *Pilobolus* sporangiophores appeared. Al-

though the length of the sporangiophores varied considerably, it was apparent that only one species was present. Successive crops of sporangiophores were produced over a period of several days.

Since mature sporangia are shot away from the sporangiophores towards the light source, they could be caught on a poured agar Petri dish by inverting the bottom of the plate between the sporangiophores and the light. Sporangia are typically discharged each morning. In this manner many sporangia were collected on the surface of dung agar. Spore germination was accomplished by placing a single sporangium in a hanging drop to which a commercial preparation of pancreatin had been added. The germinating spores were then streaked out on dung agar in a Petri dish. After several hyphal-tip transfers, the *Pilobolus* was freed of contaminating bacteria and fungi. It was observed in several cases that a bacterium seemed to stimulate the production of trophocysts.

The *Pilobolus* species, when isolated, showed mycelial growth just below the surface of the agar. No aerial mycelium is ever formed except for the transitory sporangiophores which collapse at the time of sporangial discharge. Fruiting is characteristically around the margin of the Petri dish as if exhaustion of a nutrient brought about spore production. However, fruiting may occur over the entire surface of the plate. Fruiting is preceded by the formation of trophocysts, which are cut off by a septum from the coenocytic mycelium and can be used as indicators of the time when sporangial formation is about to occur. Microscopically the trophocysts are yellow and turnip-shaped. The sporangiophores are variable in height. The sporangia, at the time of discharge, are black, smooth-walled, without a reticulate sporangial wall, and the majority measure 148–220 μ in diameter. The sporangiospores are imbedded in a gelatinous matrix in the sporangia making their separation difficult. The sporangiospores from freshly discharged sporangia are ovoid, yellow, thin-walled, and measure 11–16 $\times$ 6–9.8 μ. From the key in Buller (**4**) and in Hesseltine (**6**) this *Pilobolus* species is *Pilobolus kleinii* van Tieghem.

Two stock solutions were used in the culture medium.

I. Dung decoction was prepared by suspending 125 g. of Bovung [1] (a commercial dried cow manure) in 1000 ml. of tap water for 2

[1] Walker-Gordon Laboratory Co.

to 3 hours and successively filtering through 2 thicknesses of cotton gauze and filter paper.

II. Oatmeal extract was prepared by boiling a 2 per cent oatmeal in tap water for five minutes and straining the hot solution through 2 thicknesses of cotton gauze.

To prepare the medium, 15 g. of crude agar were added to a mixture of 500 ml. of dung decoction and 500 ml. of oatmeal extract, and this was autoclaved for 15 minutes at 15 lbs. pressure. It is important not to overautoclave this solution. The above dung agar is referred to as the stock culture medium or as dung agar, and was used routinely throughout the work. It was also possible to grow *Pilobolus* cultures on McVickar's dung agar (**9**) and also on asparagine dextrose agar to which some of the above dung decoction had been added. These latter two media have the advantage of being clear agars, enabling one better to observe the mycelium, trophocysts, and the initiation of sporangiophores. It is important to be certain that the pH of the agar remains above 7. McVickar's media, as stated by the author, was adjusted to pH 6.5, which is below the optimum for *Pilobolus kleinii;* this may explain the abnormal sporangiophores which McVickar described, and on which he based 3 new species of *Pilobolus.*

DEMONSTRATION OF A NEW GROWTH FACTOR AND ITS ASSAY

Once a pure culture of the fungus had been established, it was tested on a defined basal medium. The test medium was prepared by adding 10 ml. of sterile 3 per cent Difco Bacto agar solution to 10 ml. of sterile basal medium (TABLE I) in a Petri plate. Since spore inoculum was not feasible, blocks of dung agar removed from the margin of a growing *Pilobolus* colony were cut out and used as inoculum.

When this was done, no growth occurred. When Petri dishes with this basal medium plus dung decoction were tried, growth resulted. The same results were obtained when the synthetic agar for *Mucors* with thiamine added was tried (**6**).

The growth factor for *Pilobolus* has been named Coprogen. It has been isolated as a pure crystalline compound hitherto undescribed, the details of which will be reported at a later date.

TABLE I

COMPOSITION OF DOUBLE STRENGTH BASAL MEDIUM (10)

HCl-hydrolyzed casein	2.5 ml*	Folic acid	12 μg.
Sodium acetate	3.0 g.	Biotin	1.2 μg.
		B_{12}	2.4 μg.
L cystine	50.0 mg.		
DL tryptophane	100.0 mg.	Speakman Salts solution A	2.5 ml.(10)
Adenine	5.0 mg.	Speakman Salts solution B	2.5 ml.(10)
Guanine	5.0 mg.	Distilled water to pH adjusted to 7.6	250 ml.
Uracil	5.0 mg.		
Xanthine	5.0 mg.		
Thymine	5.0 mg.		
Thiamine	500 μg.		
Pyridoxine	100 μg.		
Ca pantothenate	100 μg.		
Riboflavin	200 μg.		
Nicotinic acid	500 μg.		
Choline	500 μg.		
Inositol	200 μg.		
PABA	200 μg.		

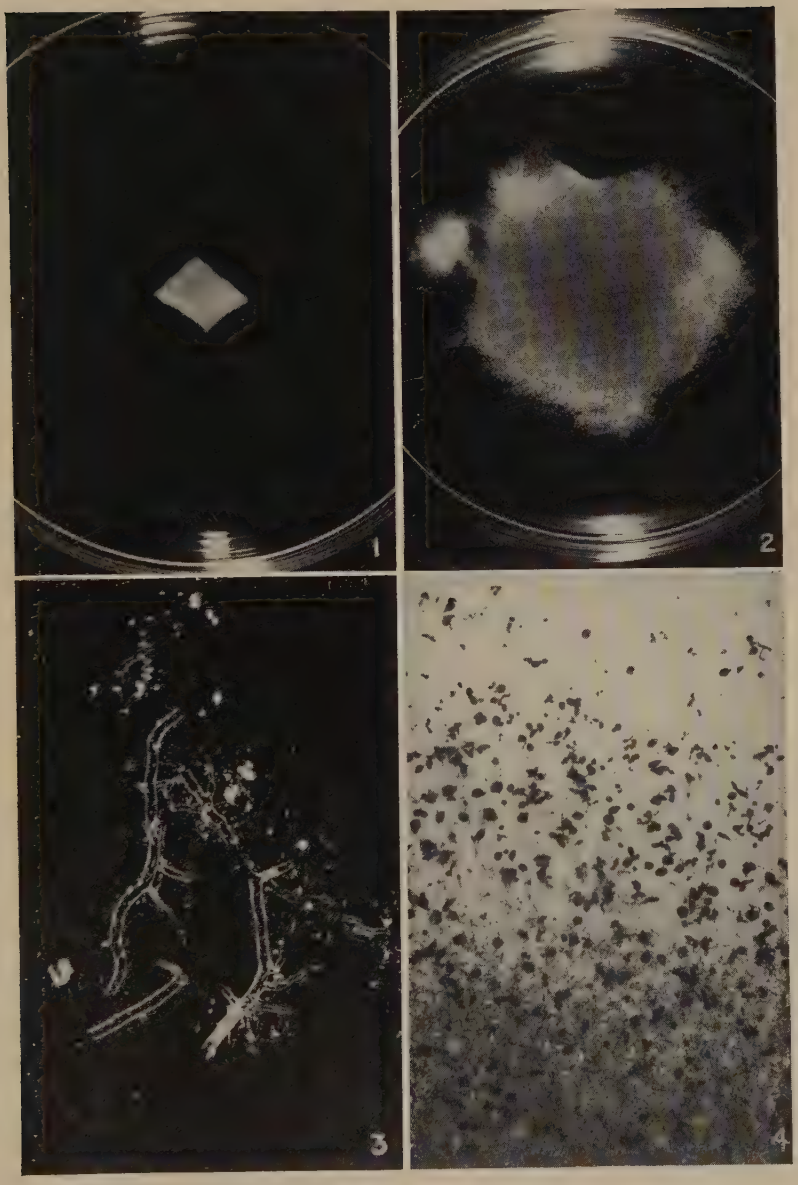
When used for liquid assay, 10 ml. of medium were added to 250 ml. flask. The test solution was added and volume adjusted to 20 ml.

* 1 ml. ≅ 100 mg. of casein.

GROWTH IN LIQUID MEDIA

In an attempt to grow *Pilobolus* in liquid media, 25 ml. of dung decoction was sterilized in 500 ml. Erlenmeyer flasks. These flasks were inoculated with small blocks of dung agar from Petri plates containing the mycelium of *Pilobolus kleinii,* which immediately settled beneath the surface of the liquid. In 3 to 4 days a dense felt of mycelium was formed which, unlike most fungi, remained just below the surface of the liquid. From this felt of mycelium an abundant crop of sporangiophores was produced principally on a single day, although some were formed on several successive days. In many cases the sporangia were yellow instead of the usual black color, but later liquid cultures have indicated that normal sporangial production occurred. The sporangia were forcibly discharged just as they were on dung. As far as we are aware, this is the first time *Pilobolus* has been grown in liquid media. The mats were solid and could be readily lifted intact from the flasks.

Since the stock dung agar medium could be autoclaved, it was thought that the liquid assay of the factor in dung could be accomplished by autoclaving the basal medium with the fraction under study. Accordingly Erlenmeyer flasks were prepared with 10 ml. of the medium shown in TABLE I. Sterilization was accomplished



FIGS. 1–4. 1. Block of dung agar containing mycelium of *Pilobolus* used to inoculate the liquid assay. 2. The mycelial pad from an assay flask in which Coprogen was present. 3. Fragments of mycelium of *Pilobolus kleinii* after treatment with a Waring blender. 4. Portion of the mycelial pad somewhat magnified from FIG. 2.

by autoclaving for 15 minutes at 15 pounds pressure. After cooling, Seitz-filtered dung decoction and sterile distilled water were added to bring the volume to 20 ml., and the flasks were inoculated with blocks of agar (FIG. 1) containing some of the *Pilobolus* mycelium from a 3-day-old stock culture. These were then incubated at 28° C. for six days. The mycelium (FIGS. 2, 4) was lifted from the medium, the excess liquid was expressed, and the pad was dried at 110° C. for 16 hours. The results in TABLE II indicate a quantitative response.

TABLE II

ASSAY OF COPROGEN IN DUNG EXTRACT

Quantities of media in ml.			Dry weights of mycelium in mg. at 6 days
Dung decoction	Distilled water	Basal medium	
1	9	10	1.4
2	8	10	3.1
3	7	10	7.2
4	6	10	6.1
5	5	10	18.9
6	4	10	26.1
7	3	10	25.8
8	2	10	29.8
9	1	10	36.0
10	0	10	36.7

FACTORS INFLUENCING GROWTH OF PILOBOLUS

Stock dung agar plates were inoculated with blocks of agar containing *Pilobolus* mycelium and were incubated at four temperatures. There was essentially no growth at 36° C. except for a few filaments not more than 2 to 3 mm. in length; at 32° C. in the same period of time the plates were covered with mycelium but no trophocysts or sporangia were seen. In the same period at 28° C. growth covered the entire plates, and large numbers of trophocysts and sporangiophores were produced. At 24° C. growth was slower, but trophocysts were abundantly formed though fruiting had not yet been initiated at the time the plates were read.

An experiment was also set up to determine the pH range of growth for the *Pilobolus kleinii* isolate. Ten ml. of distilled water containing varying quantities of NaOH or HCl was sterilized in 250 ml. Erlenmeyer flasks, and then 10 ml. of Seitz-filtered dung

decoction was added. Each pH level was made up in sets of 4. One flask of each series was sacrificed before inoculating to determine its pH. The remaining three flasks were inoculated with small blocks of dung agar containing mycelium, and incubated at room temperature for 4 days. It will be noted that growth occurred on the alkaline side even at pH 10 but that the *Pilobolus* tended to bring the pH down to approximately 8–8.5. An acid pH of 6 or below completely prevented growth.

TABLE III

EFFECT OF PH ON GROWTH OF *PILOBOLUS KLEINII*

Initial pH	Final pH			Weight of mycelium in mg.		
	1	2	3	1	2	3
Uninoculated control 6.58						
3.2	3.3	3.3	3.2	*	*	*
4.1	3.9	3.8	3.9	*	*	*
5.3	5.1	5.2	5.2	*	*	*
6.2	6.1	6.4	7.3	*	*	4.1
7.2	7.9	8.0	8.0	5.3	4.7	6.4
8.0	8.1	8.2	8.1	4.7	4.7	4.9
8.9	8.2	8.3	8.3	3.4	3.5	4.3
10.1	8.6	8.6	8.6	4.3	3.9	4.3

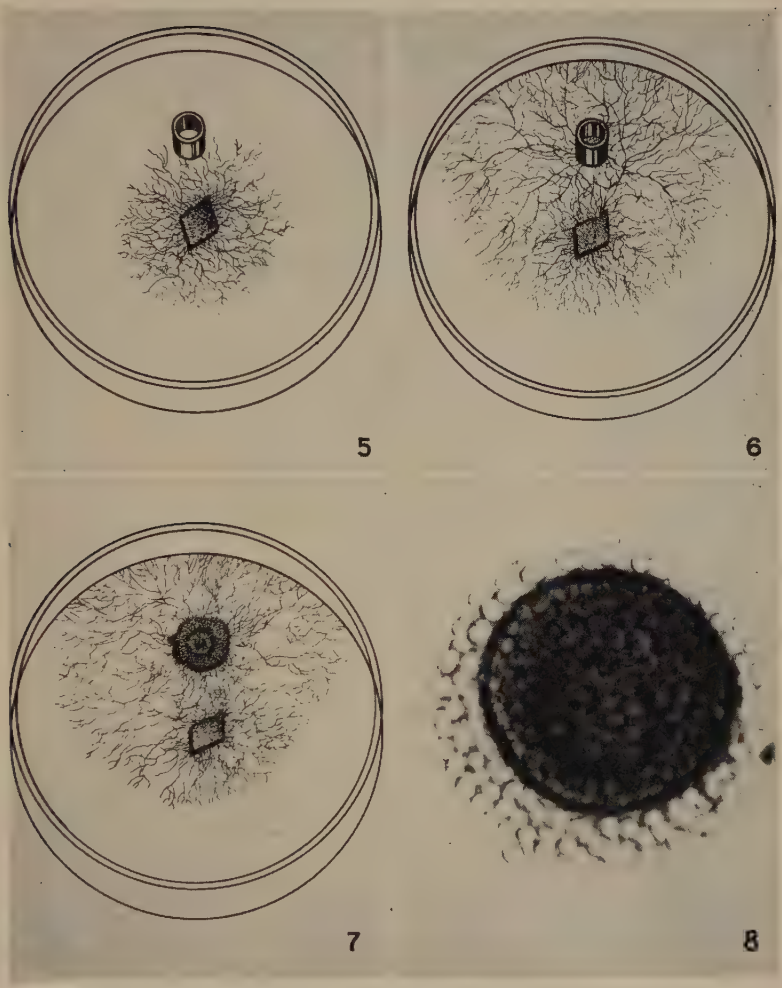
* Indicates no growth.

In any liquid assay it is desirable to use spores as inoculum (FIG. 8). With *Pilobolus* this was immediately found to be difficult because the spores are shot away from the surface of the medium against the sides and top of the container; they are contained in a gelatinous matrix which makes it difficult to separate them into a uniform spore inoculum; the conditions for consistent spore germination are unknown. A number of attempts were made to obtain spore germination. Ordinarily the spores from discharged sporangia fail to germinate when placed in tap water (neutral or acidified) or in dung decoction. Pretreatment in tap water of approximately 0.005 N HCl did not enhance germination. Spores suspended in tap water or acidified tap water for 24 hours at 37° C. showed no growth. Believing that perhaps a factor in the growing mycelium might have an effect, Petri dish lids which had many sporangia on them were flooded with the broth from cultures of actively growing *Pilobolus*. When these spores were examined, no

germ tubes were demonstrated. On several occasions, when suspended in alkaline dung decoction, alkaline trypsin solutions, or pancreatin dung decoction solutions, nearly 100 per cent germination resulted. However, even under these conditions a high percent of germination could not be predicted. Bacteria present in the enzyme solutions did not rule out their possible action. The age of the spores also seemed to be important. Because of the erratic nature of spore germination, this method of inoculation was abandoned, and the agar blocks were used in its place. Mycelial pads treated with a Waring blender did not result in a satisfactory inoculum because coenocytic Phycomycetes, unlike the other classes of fungi, possess no cross-walls or at most only a few; consequently, the breaking of the mycelium results in the cytoplasm flowing out into the medium with the resultant death of the fragment (FIG. 3).

PLATE ASSAY OF COPROGEN

Although the dry weight assay gives quantitative results, it seemed desirable to develop a more rapid and easier means of assay. Accordingly, the following procedure was found to give both rapid and reliable readings. Agar plates were prepared with the basal medium as previously described for the demonstration of the factor. Each plate was inoculated with a block of fresh stock dung agar containing actively growing mycelium of *Pilobolus*. The plates were incubated for 3–4 days at which time Coprogen was depleted from the block, and the *Pilobolus* mycelium had grown out for a distance of 1.5 to 3 cm. The edges of the colony were ragged and not too clearly defined because the main fungus filaments often grow farther than some of the shorter branched feeder filaments. The material to be tested was placed either in a paper disc or preferably in a cylinder on the agar at the edge of the colony. A typical assay is shown in FIG. 5. FIG. 6 shows a strong positive response. Where none of the factor was present, no further growth occurred. Readings can be made at 24 hours, but the final reading should be made at 48 or even 72 hours in order to check borderline cases. Certain conditions must be followed to make the assay reliable.



FIGS. 5–8. 5. Schematic diagram of the plate assay at the time the unknown solution is added to the cylinder. 6. A positive response to Coprogen of *Pilobolus kleinii* on an assay plate. 7. Positive response with a microorganism to Coprogen on the plate assay. 8. A sporangium of *Pilobolus kleinii* showing the sporangial wall and sporangiospores held within a gelatinous matrix.

1. The plates after inoculation must stand until the *Pilobolus* colony has reached its maximum growth diameter.

2. The pH of the basal medium must be above 7.

TABLE IV

ORGANISMS TESTED FOR THE PRODUCTION OF COPROGEN

Organism	No. of cultures tested	Response
Sarcina lutea	1	+
Spore-forming bacilli	25	−
Lactic bacteria freshly isolated from intestinal tract of a chicken	10	−
Red pigmented actinomycete	1	+
Pink yeast	1	−
Streptomyces A 54	1	−
Streptomyces A 105	1	+
Streptomyces A 120	1	+
Streptomyces A 37	1	−
Streptomyces A 88	1	−
Streptomyces A 307	1	−
Streptomyces A 81	1	+
Streptomyces A 247	1	+
Streptomyces A 153	1	−
Streptomyces aureofaciens A–377	1	−
Streptomyces rimosus	1	−
Saccharomyces cerviseae	1	−
Saccharomyces pyriformis	1	−
Saccharomyces fragilis	1	−
Ashbya gossypii	1	−
Candida pseudotropicalis	1	−
Unidentified yeasts	4	−
Torulopsis utilis	1	−
Proteus vulgaris	1	−
Alcaligenes bookeri	1	+
Escherichia coli	1	−
Staphylococcus aureus	1	−
Aspergillus carbonarius	1	+
Aspergillus nidulans	1	−
Penicillium sp.	1	+
Mortierella vinaca	1	−
Ustilago zeae	1	+

A + sign indicates the presence of Coprogen; a − sign indicates the absence of Coprogen by plate assay.

3. The cylinders containing the liquid to be assayed should always be placed with the edge of the cylinder just against the margin of the *Pilobolus* colony in order that a sample containing a small amount of the factor will show its activity.

4. Positive readings on questionable samples must not be made before the third day.

5. A positive reading can be made only when the fungus mycelium has grown under the cylinder and appears beyond it. In

strongly positive samples the mycelium will be heavy near the cup and grow to the side of the Petri dish.

6. No more than 3 samples should be placed on a plate.

With this type of assay it was possible to substitute for the assay cylinder a placement of a mold or bacterium to determine its activity (FIG. 7). By using such an assay a number of organisms were found which produced Coprogen. These are shown in TABLE IV.

NUTRITIONAL REQUIREMENTS OF THE GENUS PILOBOLUS

All of the species besides *P. kleinii* so far tested have shown an absolute requirement for this factor. These are summarized in TABLE V.

TABLE V

SPECIES OF *PILOBOLUS* TESTED AND FOUND TO HAVE AN ABSOLUTE REQUIREMENT OF COPROGEN

Species		Source
P. umbonatus (3 isolates)	deer dung	Tuxedo Park, New York
P. longipes	horse dung	Pearl River, New York
P. roridus (2 isolates)	horse dung	Pearl River, New York
P. kleinii	horse dung	Pearl River, New York

Since it had been the experience of one of us (6) that nearly all members of the order Mucorales would grow in a medium in which asparagine was the nitrogen source, a series of basal media were prepared in which asparagine was the principal nitrogous component. When these media containing vitamins and including Coprogen were tested, growth was as good as with the basal medium given in TABLE I. A further study of the omission of various vitamins indicated a definite B_1 requirement. Consequently, a simplified basal medium was devised which had the following composition: asparagine 2 gms., Speakman salts solutions A and B 2.5 ml. of each, glucose 10 gms., sodium acetate 6 gms., and 1000 ml. of distilled water.

Using this simplified formula, *Pilobolus kleinii* was carried through 10 successive transfers on this medium with the addition of highly purified preparations of Coprogen. The 10 serial transfers were carried through in agar plates using hyphal inoculum.

Further papers on the nutrition (11), methods of isolation, and properties of the pure Coprogen will appear elsewhere.

ACKNOWLEDGMENTS

The writers wish to thank Miss Marie Hauck for certain technical assistance. Thanks are also due to Mr. Arthur A. Jansson and Mr. Leslie McWilliam for preparing the illustrations.

SUMMARY

1. A factor necessary for the growth of *Pilobolus kleinii, P. longipes, P. umbonatus,* and *P. roridus* has been demonstrated.

2. This factor has been named Coprogen.

3. A stock medium consisting of dung decoction and oatmeal extract has been described for carrying species of *Pilobolus.*

4. A block inoculum assay has been developed for the assay of Coprogen in agar in Petri dishes.

5. The pH for maximum growth has been shown to be above 7.

6. Growth of *Pilobolus kleinii* in liquid media has been made possible resulting in a liquid assay that is both quantitative and qualitative.

7. Certain aspects of spore germination have been studied in the genus *Pilobolus.*

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A RELATION BETWEEN CELL MULTIPLICATION AND ALCOHOL TOLERANCE IN YEASTS [1]

James R. Troyer

(with 4 figures)

The cessation of many physiological activities of a microorganism despite the presence of an ample supply of nutrients and otherwise favorable environmental conditions has often been ascribed to "staling," a condition presumed to result from the inhibitory action of progressively accumulating products of metabolism. When the "staling" effect is manifested in industrial processes involving the biosynthesis of economically valuable substances, a knowledge of the mechanisms operative is obviously desirable. In the case of the commercial production of ethanol by yeast fermentation, the most significant metabolic product in this respect is that which is desired in the greatest quantities—the alcohol itself. The problem has been to develop methods of culture which afford increased yields by obviating the "staling" action.

Numerous investigators have from time to time measured the ethanol concentrations at which various fermentations cease, but the first systematic comparative survey of a number of yeasts was conducted by Gray (**1941**): from glucose utilization data obtained under reproducible conditions he defined alcohol tolerance as "the maximum percentage of alcohol (by weight) at which the percentage glucose utilization is no more than 1% below the percentage glucose utilization in the control flask of the same series." He was thus able to assign arbitrary alcohol tolerance values to each

[1] Paper No. 544 from the Dept. of Botany and Plant Pathology of the Ohio State University; based largely on a thesis submitted to the Graduate School of that institution in partial fulfillment of the requirements for the degree Master of Science. The generous counsel of Dr. W. D. Gray is gratefully acknowledged.

of 21 yeasts tested. He also described a lowering of these values at an elevated temperature and noted that measurement of carbon dioxide production in the presence of ethanol affords a convenient method of estimating the alcohol tolerance of a particular yeast. In other studies (**1946**, **1948**) Gray found a definite relationship between the amount of reserve material accumulated by a yeast and its alcohol tolerance: he reported that fat content especially varies inversely with alcohol tolerance.

The values derived by Gray, based as they are on rates of glucose utilization or carbon dioxide evolution, are empirical quantities of considerable practical utility. But their formulation indicates nothing concerning the manner in which ethanol is operative in effecting the observed reductions in glucose utilization. In Gray's experiments yeasts were cultured in a medium capable of supporting growth; since the measurements were made on a culture basis, the lowered glucose utilization rates might have resulted from effects of ethanol on (1) systems participating in carbohydrate breakdown, (2) cell multiplication (fewer cells might be operative), or (3) both sets of processes. Any attempt to elucidate the nature of the ethanol inhibition must first involve a consideration of these possibilities.

In this report an attempt is made to demonstrate that lowered population levels may largely account for reductions in glucose utilization per culture under the experimental conditions imposed by Gray.

MATERIALS AND METHODS

Six yeasts were employed; these are listed with the numbers under which they are cataloged in the stock culture collection of the Dept. of Botany and Plant Pathology of the Ohio State University:

1. *Saccharomyces cerevisiae* Hansen. D.C.L. Strain.
3. *S. cerevisiae* Hansen. R Strain.
20. *Hansenula anomala* (Hansen) Sydow.
22. *H. anomala* (Hansen) Sydow.
28. *S. cerevisiae* Hansen. Rasse M Strain.
31. *S. cerevisiae* Hansen. Brown-Forman Strain.

Five of these yeasts were studied by Gray (**1941**, **1946**, **1948**) and had been obtained from the collection of Joseph E. Seagram and Sons, Inc. Yeast *22* was secured from Dr. E. M. Mrak of the University of California under the designation *Hansenula anomala* var. *sphaerica* (Naegeli) Dekker; in conformity with the recent study of this genus by Wickerham (**1951**), however, the varietal name is not retained in this report.

A liquid glucose-phosphate-yeast extract medium (Gray, **1946**), denoted "standard," was used: 12.50 g. D-glucose, 6.25 g. KH_2PO_4, 8.75 g. Difco Yeast Extract, and distilled water to make 1 liter. Since inoculum, ethanol, and distilled water were later added to portions of this medium at the rate of four volumes of medium to one volume of additional liquid, the final concentrations in the culture vessels were 1.0 per cent D-glucose, 0.5 per cent KH_2PO_4, and 0.7 per cent yeast extract. The initial pH was adjusted to 4.4–4.6 (before autoclaving), measurements being made with a Beckman line-operated glass electrode.

For the preparation of inoculum a medium of similar type was used: 100.0 g. D-glucose, 5.0 g. KH_2PO_4, 7.0 g. Difco Yeast Extract, and distilled water to 1 liter. Yeasts from stock cultures carried in slant tubes (standard medium plus agar) were transferred to sterile inoculum medium contained in 40-ml. graduated centrifuge tubes; such cultures were incubated without shaking at 30° C for 24 hours. For inoculum designated "aerated" sterile 125-ml. portions of inoculum medium in 400-ml. bottles were seeded with yeast; air was passed from a compressed air line through sterile cotton and small-bore glass tubing to the bottoms of the culture bottles. Aerated yeasts were incubated 24 hours at room temperature (28–31° C).

To effect inoculation, cells were centrifuged out of inoculum cultures, washed with sterile distilled water, and resuspended in sterile M/15 KH_2PO_4 solution to the desired concentration. Proper amounts of suspension were added to culture vessels through sterile graduated pipettes.

Some fermentations were run in 50-ml. Erlenmeyer flasks, each containing a final volume of 25 ml. of liquid: 20 ml. of standard medium, 0.5 ml. of inoculum suspension, and 4.5 ml. of ethanol and

distilled water in varying proportions. Other fermentations were conducted in one-ounce soft-glass French square bottles, each with a final volume of 10 ml.: 8 ml. of standard medium, 0.2 ml. of inoculum suspension, and 1.8 ml. of ethanol and distilled water. Medium and water were placed first in the flasks or bottles, which were stoppered with cotton and autoclaved 20 minutes at 15 pounds gauge pressure; the ethanol was then added aseptically.

In all experiments except that concerned with the effect of temperature on cell numbers the cultures were maintained without shaking at 30° C; in the latter instance one series was held at 35° C, also without shaking.

Estimations of cell numbers were made turbidimetrically or by direct count. In the second case a Spencer Bright-Line Haemacytometer was used, and all well-defined buds which were more than one-fourth the size of the parent cell were counted. In the turbidimetric measurements cultures were shaken with glass beads to insure uniform suspensions, then allowed to stand one minute for dissipation of undissolved gases. Optical densities were read in a Klett-Summerson photoelectric colorimeter with a No. 42 blue filter against a blank of uninoculated medium; cell concentrations were calculated through the use of experimentally determined conversion factors.

For Yeasts 1, 3, 22, and 31 such turbidity measurements agreed rather well with direct counts; hence, these yeasts were employed in experiments in which populations were measured in this manner. As has been reported previously, attempts to correlate such measurements using Yeast 20 were fruitless because of great variability in size and shape of the cells (Kuehner, **1951**). In the present study, however, variations in results obtained with the closely related Yeast 22, while near the general limitations of the method, were not of sufficient magnitude to preclude use of this strain, provided care was exercised.

Glucose concentrations were estimated by the method of Stiles, Peterson, and Fred (**1926**).

EXPERIMENTAL RESULTS

Numbers of cells formed in ethanol. In these experiments Yeasts 1, 3, 20, 22, 28, and 31 were employed. For each yeast two

replicate series of fourteen 50-ml. flasks containing various initial amounts of ethanol were prepared. Twenty-four hours after inoculation haemacytometer counts were made, two per flask, and the values obtained for duplicate flasks averaged. These results are presented in TABLE I: the figures represent the average numbers of cells formed (final cells minus initial cells) as millions per milliliter for each alcohol concentration. In order that results for

TABLE I

AVERAGE NUMBERS OF CELLS FORMED (FINAL MINUS INITIAL) IN 24 HOURS BY SIX YEASTS IN CULTURES CONTAINING ETHANOL. YEASTS ARE ARRANGED FROM LEFT TO RIGHT IN ORDER OF INCREASING ALCOHOL TOLERANCE. (INITIAL CELLS: 1×10^6 PER ML.)

Initial EtOH	Yeast					
	20	22	1	28	3	31
mg./ml.	millions of cells per ml.					
0.0	62	71	31	60	38	59
29.4	41	49	32	57	36	62
36.8	23	44	24	65	41	58
44.1	6	33	22	52	33	57
51.5	0	26	15	50	30	52
58.8	0	3	8	27	26	33
66.2	0	1	2	1	3	3
73.6	0	1	1	0	0	3
81.0	0	0	0	0	0	0
88.3	0	1	0	0	0	0
95.7	0	0	0	1	0	0
103.0	0	0	0	0	0	0
117.7	—	0	0	0	—	0
132.4	—	1	0	0	—	1

the six yeasts may be compared, these averages are presented graphically in FIG. 1 as percentages of the number of cells obtained in the control flasks to which no alcohol was added.

In each case the number of cells formed in 24 hours decreased as the ethanol concentration increased, although not linearly. The reduction in population at a given alcohol concentration was much greater with some yeasts than with others. When the five (disregarding Yeast 22) which were studied by Gray (**1941**) are arranged on the basis of the ethanol concentrations at which equivalent reductions in the numbers of cells formed occur, the order is $20 < 1 < 28 < 3 < 31$. Such ranking may be seen easily from

FIG. 1. This is exactly the order which was reported by Gray, as based on the ethanol concentrations at which equivalent reductions in glucose utilization were observed—that is, as based on alcohol tolerance as defined by him.

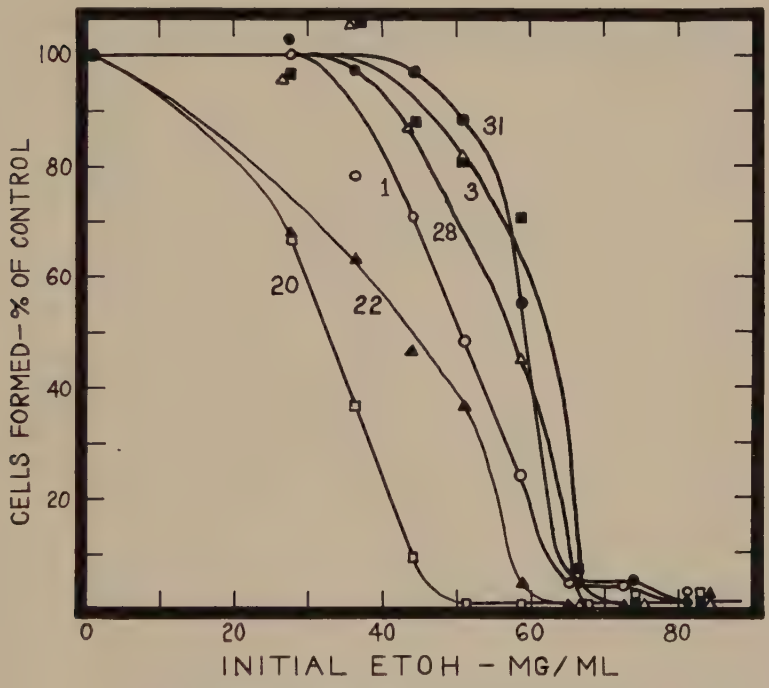

FIG. 1. Average numbers of cells formed (final minus initial) in 24 hours by six yeasts in cultures containing initial ethanol. Cell numbers are expressed as percentages of the results for the control flasks to which no ethanol was added.

Yeast 22, which was not employed by Gray (his Yeast 22 was a strain of *Saccharomyces cerevisiae* Hansen), falls in the above series between Yeast 20 and Yeast 1; with this strain of *Hansenula anomala* (Hansen) Sydow the reductions in 24-hour populations in the presence of ethanol were large, but not as great as those found for the related Yeast 20.

Effect of temperature. Since Gray (1941) reported that Yeast 1 exhibited a lower alcohol tolerance at 35° C than at 30° C, an experiment was conducted to determine the effect of temperature on

the numbers of cells produced in 24 hours in cultures containing ethanol. Yeasts 1, 3, 22, and 31 were employed. For each yeast four replicate series of six French square bottles containing various amounts of ethyl alcohol were prepared as previously described. Two series for each yeast were held at 30° C, and two at 35° C. Twenty-four hours after inoculation the cell populations were estimated turbidimetrically and the values obtained for replicate cultures averaged.

TABLE II

EFFECT OF TEMPERATURE ON AVERAGE NUMBERS OF CELLS FORMED (FINAL MINUS INITIAL) IN 24 HOURS BY FOUR YEASTS IN CULTURES CONTAINING ETHANOL. (INITIAL CELLS: 1×10^6 PER ML.)

Yeast	Temperature	Initial EtOH, mg./ml.					
		0.0	29.4	44.1	58.8	73.6	88.3
	° C	millions of cells per ml.					
1	30	27	26	22	16	4	2
	35	25	14	9	5	5	5
3	30	31	31	28	22	6	4
	35	21	14	9	7	6	6
22	30	69	52	33	7	10	6
	35	14	8	9	10	12	10
31	30	62	62	57	49	16	5
	35	18	11	9	9	11	9

From the results presented in TABLE II it is apparent that in each case fewer cells were formed at the higher temperature than at the lower; the exceptions in cultures containing 88.3 mg. ethanol per ml. do not seem significant since at both temperatures relatively few cells were formed. It should be noted that even in the control cultures to which no ethanol was added considerably fewer cells were formed at 35° C than at 30° C. (Results for Yeast 1 are also shown in FIG. 2.)

Aerated and nonaerated inocula. Gray (1948) reported that cultures of yeast from aerated inoculum exhibited lower alcohol tolerances than cultures prepared from nonaerated inoculum. Accordingly, an experiment was conducted to determine whether differences in population levels occur under these conditions. For

each yeast used (1, 3, 22, and 31) four replicate series of six French square bottles with various initial amounts of ethanol were prepared. Inoculum was cultured for 24 hours with and without aeration. Approximately equal numbers of cells from each type of culture were used in inoculating the test bottles: for each yeast

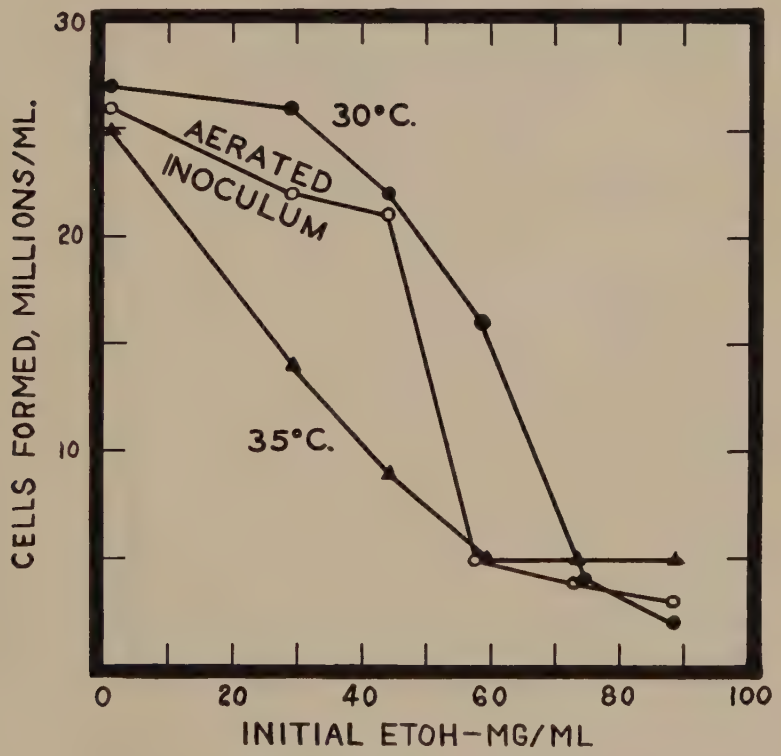

FIG. 2. Effect of temperature and aeration of inoculum on average numbers of cells formed (final minus initial) in 24 hours by Yeast 1 in the presence of ethanol. Cultures from nonaerated inoculum were maintained at 30° and 35° C, cultures from aerated inoculum at 30° C.

two series were inoculated with cells from the aerated culture and two series with cells from the nonaerated culture. After incubation for 24 hours at 30° C cell numbers were measured turbidimetrically and the values for replicate cultures averaged.

Results of this experiment are presented in TABLE III (data for Yeast 1 are also shown in FIG. 2). At almost every ethanol con-

centration fewer cells were formed by each yeast in the cultures from aerated inoculum than in those from nonaerated inoculum. Again, even when no alcohol was added initially there were differences between the numbers of cells formed in the two types of cultures; on the whole, however, these were small.

TABLE III

EFFECT OF ETHANOL ON AVERAGE NUMBERS OF CELLS FORMED (FINAL MINUS INITIAL) IN 24 HOURS BY FOUR YEASTS IN CULTURES FROM AERATED AND NONAERATED INOCULA. (INITIAL CELLS: 1×10^6 PER ML.)

Yeast	Inoculum culture method	Initial EtOH, mg./ml.					
		0.0	29.4	44.1	58.8	73.6	88.3
		millions of cells per ml.					
1	Nonaerated	27	26	22	16	4	2
	Aerated	26	22	21	5	4	3
3	Nonaerated	31	31	28	22	6	4
	Aerated	30	27	26	21	4	2
22	Nonaerated	69	52	33	7	10	6
	Aerated	59	47	15	8	8	9
31	Nonaerated	62	62	57	49	16	5
	Aerated	62	61	56	40	5	3

Time-course of multiplication in ethanol. In view of the observed reductions in 24-hour population levels described above, an attempt was made to ascertain more specifically the effects of ethanol on yeast growth by measuring the multiplication of four yeasts (1, 3, 22, and 31) over a period of 96 hours. For every yeast five sets of French square bottles, each representing a different initial ethanol concentration, were prepared; the cultures were held at 30° C throughout the experimental period. At various times the cell population in one culture from each set was measured turbidimetrically; results are expressed as averages of duplicate determinations, between which close agreement was found. Data from this experiment are presented in TABLE IV; results for Yeast 1 are also summarized graphically in FIG. 3.

Although the yeasts employed in this study differ from bacteria in manner of cell division and the growth pattern of the yeasts does not follow exactly that of bacteria, it is believed that the differ-

ences are not so great as to preclude analysis in terms of concepts developed by the bacteriologists. Growth curves obtained by plotting the data of TABLE IV are of the familiar type; in particular, in the three series of cultures having the lowest ethanol concentrations an exponential period of multiplication was closely approximated

TABLE IV

GROWTH DURING A 96-HOUR PERIOD OF FOUR YEASTS IN CULTURES CONTAINING INITIAL ETHANOL

Yeast	Initial EtOH	Hours after inoculation									
		0	3	6	9	22	33	47	54	72	96
	mg./ml.	millions of cells per ml.									
1	0.0	1	1	7	16	26	27	31	32	31	32
	29.4	1	1	3	10	26	26	28	29	31	32
	58.8	1	1	2	2	18	17	21	22	22	23
	88.3	1	1	2	2	3	4	4	3	5	7
	117.7	1	2	2	2	2	3	3	3	.3	4
3	0.0	1	3	9	19	29	30	35	36	38	39
	29.4	1	3	6	16	29	29	34	35	36	38
	58.8	1	2	4	4	23	25	30	32	32	35
	88.3	1	3	3	3	3	3	4	13	27	31
	117.7	1	3	4	3	3	4	5	5	5	5
22	0.0	1	3	7	11	48	—	87	106	123	125
	29.4	1	3	6	7	42	60	80	89	104	124
	58.8	1	3	3	2	5	9	23	25	40	39
	88.3	3	4	5	5	5	5	7	8	8	12
	117.7	3	4	4	4	4	4	6	4	7	6
31	0.0	2	3	9	25	58	58	67	68	71	70
	29.4	2	3	5	16	57	57	66	66	70	68
	58.8	2	3	4	5	28	49	55	57	63	64
	88.3	2	4	5	4	6	7	9	30	53	57
	117.7	2	4	4	4	6	6	7	6	8	10

by each yeast. Hence certain growth constants described by Monod (1949) have been calculated. These constants, values of which are presented in TABLE V, may be briefly described.

Total Growth,

$$G = x_{max} - x_0,$$

expresses the difference between the initial (x_0) and maximum (x_{max}) cell concentrations (here cells per unit volume are used, rather than the dry weight per unit volume preferred by Monod). In every case as the ethanol concentration increases, the value of G

decreases, although the latter is certainly not a linear function of alcohol concentration (TABLE V). Also, the degree to which G for a yeast is decreased at a given concentration bears a relationship

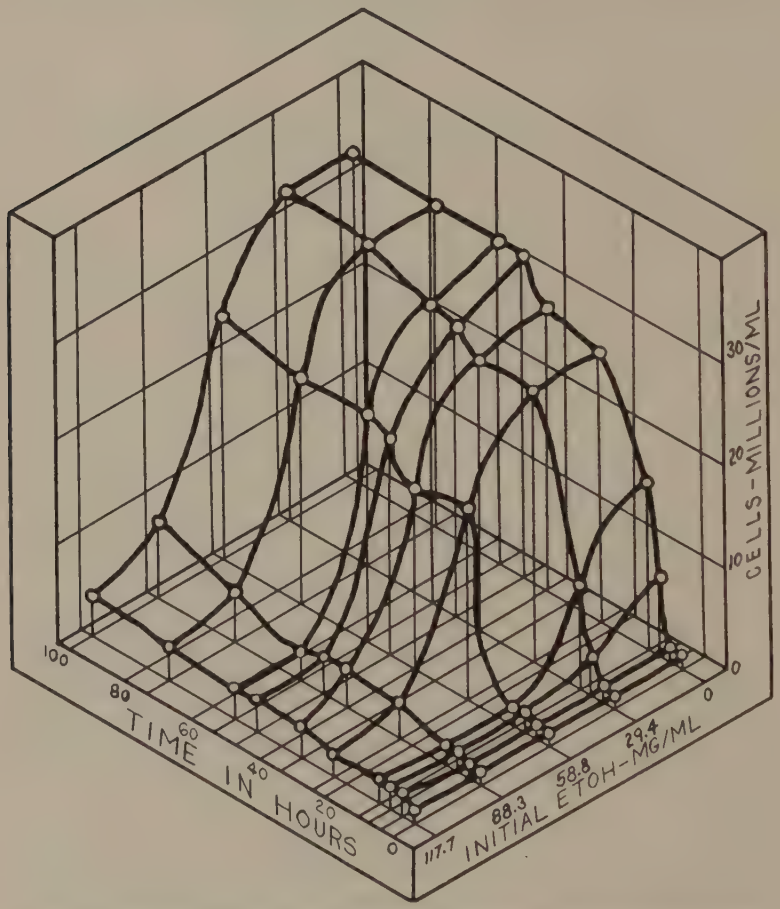

FIG. 3. Growth of Yeast 1 as a function of initial ethanol concentration and time.

to the alcohol tolerance of that yeast, as has been shown for 24-hour cultures in experiments described earlier.

Exponential Multiplication Rate is expressed by the equation

$$R = \frac{\log_2 x_2 - \log_2 x_1}{t_2 - t_1},$$

where $t_2 - t_1$ is any time interval within the exponential period of growth and x_1 and x_2 represent the numbers of cells at the beginning and end of that interval. In this study no exponential phase was observed for any yeast at ethanol concentrations of 88.3 and 117.7 mg. per ml., but R-values have been calculated for each yeast in the other three concentrations (TABLE V). From the results it appears that ethanol may have the effect of decreasing the exponential multiplication rate: only with Yeast 31 was this trend not followed exactly.

TABLE V

CALCULATED GROWTH CONSTANTS FOR 96-HOUR CULTURES OF FOUR YEASTS IN MEDIUM CONTAINING ETHANOL. (G = TOTAL GROWTH; R = EXPONENTIAL MULTIPLICATION RATE)

Yeast	Constant	Initial EtOH, mg./ml.				
		0.0	29.4	58.8	88.3	117.7
1	G	31	31	22	6	3
	R	0.77	0.52	0.43	—	—
3	G	38	37	34	30	4
	R	0.52	0.42	0.32	—	—
22	G	124	123	39	9	3
	R	0.43	0.42	0.10	—	—
31	G	69	68	62	55	8
	R	0.54	0.60	0.22	—	—

Two lag constants, the Lag Time (Lodge and Hinshelwood, 1943) and the Growth Lag (Monod) were also calculated; in some cases there was a regular increase in the values of these lag constants with increasing ethanol concentration. Among different yeasts the results varied considerably, however, and they are therefore not reported. From TABLE IV it is nevertheless obvious that an increase in lag was generally observed.

Evidently the growth of yeasts may be variously affected by ethanol in the medium. In this study the total number of cells formed varied inversely, although not proportionally, with the amount of alcohol present. This may have resulted largely through the reduction in multiplication rate during the exponential phase (and eventual elimination of this period), as well as from a possible

increase in lag. Other factors, such as the duration of the exponential phase, if there is one, and the rate of decrease in multiplication rate as growth tapers off are probably operative.

Cell numbers and glucose utilization. Reductions in yeast populations accompany the reductions in glucose utilization in the presence of ethanol reported by Gray for various experimental condi-

TABLE VI

AVERAGE NUMBERS OF CELLS FORMED (FINAL MINUS INITIAL) AND AMOUNTS OF GLUCOSE UTILIZED IN 24 HOURS BY SIX YEASTS IN CULTURES CONTAINING ETHANOL. (INITIAL CELLS: 1×10^6 PER ML.; INITIAL GLUCOSE: 10.150–10.460 MG./ML.)

Yeast	Initial EtOH	Cells formed	Cells formed, % of control	Glucose utilized	Glucose utilized, % of control
	mg./ml.	millions per ml.	%	mg./ml.	%
1	0.0	26	100.0	10.249	100.0
	29.4	21	80.8	10.460	102.1
	44.1	18	69.2	10.237	99.9
	58.8	7	26.9	1.127	10.9
	88.3	3	11.5	0.447	4.4
3	0.0	25	100.0	9.951	100.0
	29.4	25	100.0	9.959	100.0
	44.1	19	76.0	9.922	99.7
	58.8	6	24.0	5.765	57.9
	88.3	4	16.0	0.962	9.7
22	0.0	50	100.0	9.933	100.0
	29.4	19	38.0	4.777	48.1
	44.1	3	6.0	1.362	13.7
	58.8	3	6.0	1.087	10.9
	88.3	2	4.0	0.707	7.1
31	0.0	60	100.0	9.887	100.0
	29.4	57	95.0	9.892	100.0
	44.1	51	85.0	9.889	100.0
	58.8	8	13.3	5.891	59.6
	88.3	6	10.0	0.795	8.0

tions. The question which arises is whether the reduction in glucose utilization is a result of a lower population level, or whether the smaller population may be an effect of the utilization of less glucose.

An experiment was conducted in which both cell populations and glucose utilization were measured. For each yeast used (1, 3, 22, and 31) two replicate series of 50-ml. flasks were set up with increasing amounts of ethanol in successive flasks. The cultures

were held without shaking at 30° C for 24 hours; cell concentrations were then measured turbidimetrically and residual glucose determined. For Yeast 1 two additional series were prepared and similar measurements made after 48 hours. Values of duplicate cultures were averaged; results of the 24-hour experiments are

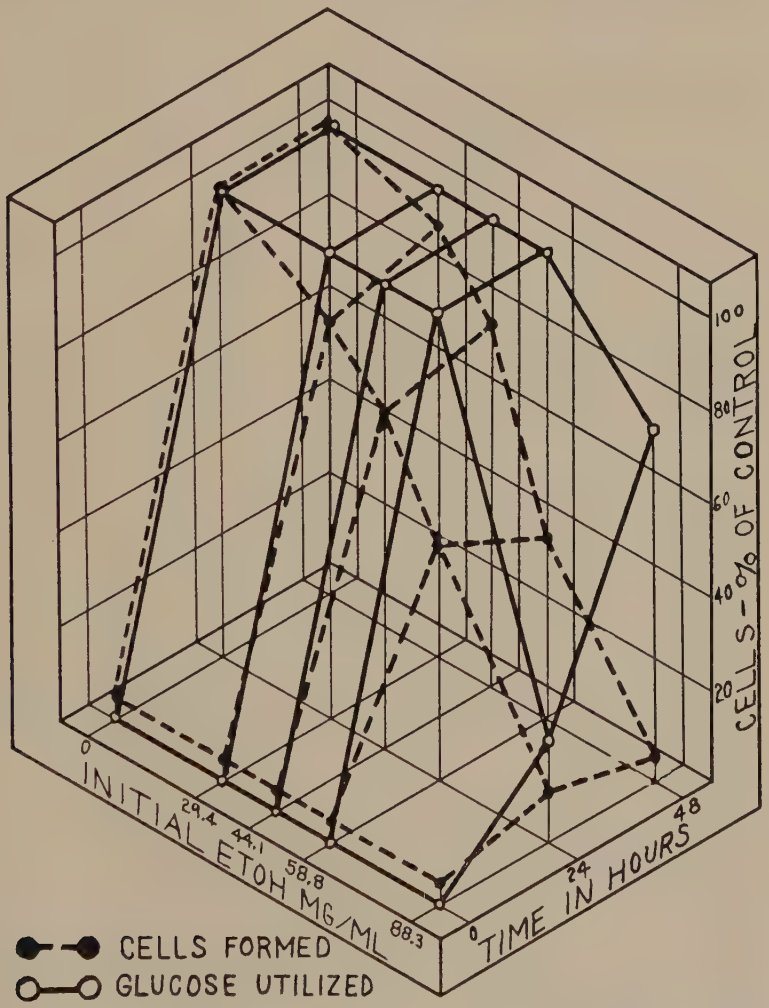

FIG. 4. Cell formation and glucose utilization in 48 hours by Yeast 1 in cultures containing initial ethanol. Numbers of cells formed and amounts of glucose utilized are expressed as percentages of the 48-hour values for control flasks to which no ethanol was added.

presented in TABLE VI, and the complete data for Yeast 1 are summarized in FIG. 4.

With these yeasts, cell populations fall off more rapidly with increasing ethanol concentration than does glucose utilization. If total growth depended only on the amount of glucose utilized, then the cells-ethanol and glucose-ethanol curves should decline sharply at nearly the same point. This, however, is not the case. Apparently reductions in cell populations in medium containing ethanol are not solely the result of decreased glucose utilization; the processes involved in cell multiplication seem to be affected at lower alchohol concentrations than are those connected with the disappearance of glucose.

DISCUSSION

The above experiments were designed to follow as closely as possible the procedures employed by Gray in his studies. From the results it is apparent that parallel reductions in cell populations accompany the reductions in glucose disappearance which he observed.

The number of cells formed by the six yeasts in graded series of cultures containing ethanol yield curves similar in shape to those reported by Gray for glucose utilization by five of the same yeasts under like conditions. Likewise, a decrease in alcohol tolerance with a rise in temperature is paralleled by reduced cell multiplication. Finally, the fact that cells from aerated and non-aerated cultures of the same yeast exhibit differences in alcohol tolerance is matched by the fact that such cultures also show differences in population levels attained in the presence of ethanol.

That ethyl alcohol is responsible for reduction in total numbers of yeast cells formed has been reported frequently—*e.g.*, by A. J. Brown (**1905**), H. T. Brown (**1914**), Richards (**1928c**), and others.

The reduction in multiplication which accompanies lower alcohol tolerance at elevated temperatures is also not unexpected: Richards (**1928a, 1928b**) reported that above 30° C the process of budding is critically affected and growth falls off rapidly. Thus, the apparently increasing effectiveness of ethanol at temperatures above

the optimum (28° C) may be explained partly by the lowered multiplication rates which occur solely as a result of the temperature rise, and perhaps partly by a shift in the equilibria of the critical reaction involving ethanol (Rahn, **1945**).

Since aerated cells accumulate more fat, Gray (**1948**) has proposed on the basis of observations of Lindegren (**1945**) that a more ready coalescence of fat globules in cells of high fat content may account for the lower alcohol tolerance of aerated cells than of nonaerated cells of a yeast strain. Lowered multiplication rates of aerated cells in ethanol might also result from such greater coalescence of fat globules.

While most workers measuring ethanol effects on yeast growth have been concerned with the total growth, H. T. Brown (**1914**) studied 100-hour growth curves and reported reductions in both total growth and multiplication rate with increasing alcohol concentration. This conclusion is borne out in the present study, since total growth declined and the value of the exponential growth rate constant generally decreased with increasing amounts of ethanol until the exponential period vanished altogether.

The question posed earlier—whether the reductions in glucose utilization noted by Gray results from the effects of ethanol on the glucose breakdown mechanism, or on cell multiplication processes, or on both systems—may be partially answered by the experimental results described here. Two facts point to the idea that growth reductions result in the utilization of less glucose per culture (rather than that a lowered rate of carbohydrate breakdown limits multiplication).

In the first place, while 24-hour cell population-ethanol curves (FIG. 1) are similar in shape to the 24-hour curves of glucose utilization-ethanol for the same yeasts (Gray, **1941**), it is noteworthy that in every case the rather abrupt change in slope characteristic of both sets of curves occurs at a lower alcohol concentration in the case of the population curves than with the glucose utilization curves. Recalculations of Gray's original data, to enable comparison, are contrasted in TABLE VII with those of the present study; with these yeasts the approximate concentrations at which the rapid drop in multiplication begins are definitely lower than those at which glucose utilization declines. Secondly,

when populations and sugar disappearance were measured in the same culture, the former were reduced more by ethanol than the latter. This situation is especially apparent from FIG. 3.

While these results are certainly not conclusive, they support the hypothesis that a limiting of cell multiplication by ethanol is initial and primary in the alcohol tolerance effect. Such a concept is in accord with the statement of Rahn and Barnes (**1933**)

TABLE VII

COMPARISON OF THE ETHANOL CONCENTRATIONS AT WHICH CHARACTERISTIC SHARP REDUCTIONS OCCUR IN GLUCOSE UTILIZATION PER CULTURE AND TOTAL NUMBERS OF CELL FORMED. (GLUCOSE UTILIZATION VALUES RECALCULATED FROM DATA OF GRAY, 1941)

Yeast	Point of sharp reduction	
	Glucose utilization	No. of cells
	mg. EtOH per ml.	
20	<37.1	<29.4
1	59.4	29.4
28	81.7	36.8
3	81.7	36.8
31	>81.7	44.1

that in yeast cells exposed to damaging agents loss of the ability to multiply preceded loss of fermentative power. It further harmonizes with the report of Brockmann and Stier (**1947a**) that in the D.C.L. Strain (Yeast 1) utilization of the glucose initially present in a culture may proceed at a fairly constant rate per culture despite concomitant changes in yeast population.

Concerning the manner in which the effects of ethanol on yeast multiplication may be in turn reflected in glucose utilization, recent research offers a clue. Experiments such as those of Brockmann and Stier (**1947b**) indicate that the enzyme systems of yeast cells usually operate at below-capacity rates. These workers hold with Meyerhof (**1944**) that if maximum glucose utilization rates are to be attained the processes of phosphorylation and dephosphorylation must be synchronized. Since about two high-energy phosphate bonds are produced for every molecule of glucose fermented, the rate of dissipation of such bonds must balance the rate of their generation (Lipmann, **1941**); otherwise the adenylic acid

system may be saturated to such a degree that it can no longer serve as a phosphate acceptor in fermentative dephosphorylations.

In general, synthesis (cell-multiplication and accumulation of reserve materials) and hydrolysis (processes involving phosphatases) are the processes by which high-energy phosphate bonds are dissipated (Potter, **1944**). The experimental data of Brockmann and Stier (**1947b**) indicate that in growing cultures of Yeast 1 accumulation of high-energy phosphate bonds is avoided through the synthetic processes incident to cell multiplication. The rate of glucose utilization (per cell) is always higher during periods of active multiplication.

Thus, while the specific mechanisms through which ethanol inhibits yeast growth are undoubtedly complex, such reactions in preventing multiplication probably result in the accumulation of high-energy phosphate bonds—the resultant combined reduction in glucose utilization per cell and per culture appearing as the phenomenon of alcohol tolerance.

SUMMARY

Studies on the multiplication of four strains of *Saccharomyces cerevisiae* Hansen and two strains of *Hansenula anomala* (Hansen) Sydow under various conditions revealed reductions in growth which parallel the lowered glucose utilization rates previously reported.

Population curves for cultures containing various amounts of ethanol resemble those for glucose utilization by the same yeasts under similar conditions. A decrease in alcohol tolerance with elevated temperature is accompanied by reduced multiplication. Cells of the same yeast from aerated and nonaerated inocula, which exhibit differences in alcohol, also attain different population levels in the presence of ethanol.

The primary effects of ethanol on yeast growth are reduction in the total number of cells formed and lowering of the exponential multiplication rate.

Results of experiments involving estimation of cell numbers and glucose utilization in the same cultures indicate that the first effect of added ethanol is probably a reduction in multiplication, with glucose utilization by the culture as a whole declining only later.

It is suggested that the decreased growth effected by ethanol results not only in fewer active cells, but also in the accumulation of high-energy phosphate bonds. These two effects, which result in a reduction in glucose utilization, could account for the occurrence of the phenomenon of alcohol tolerance.

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CULTURAL STUDIES OF DACRYMYCES ELLISII

THOMAS J. BULAT

(WITH 9 FIGURES)

In 1888, Brefeld (1) described several species of fungi belonging to the family Dacrymycetaceae in volume 7 of his Untersuchungen. He included in his description the results obtained from germinating spores of *Dacrymyces deliquescens* in water and liquid nutrient cultures. These cultures produced thin-walled hyphae upon which were borne masses of conidia. The conidia ranged from ellipsoid to obovoid and were borne on short conidiophores either singly or in groups of two or more. He reports the size of these conidia as 7–3 × 5–2 μ.

The report by Brefeld appears to be the only work done on the cultural characteristics of this group.

In May, 1951, two collections of *Dacrymyces Ellisii* were made in the same general locality in Johnson County, Iowa. These two collections were similar morphologically except for the size of their spores. One had narrow spores averaging 14.7 × 5.7 μ while the other had broad spores averaging 13.6 × 7.5 μ.

Cultures were made on malt agar from the spores of each of these collections, GWM *6425* (narrow-spored) and GWM *6426* (broad-spored). This resulted in the development of several types of colonies. At first three types of colonies were observed, all of which were bright orange in color. They were: (1) giant colonies, (2) small colonies and (3) gelatinous pustules. Later, small white colonies arose from cultures of *6426*.

The manner in which colonies arose from various areas of the substrate after single point inoculations were made suggested that the spores were being projected in some manner. To determine whether this was the case, covers were changed from petri dishes

containing active colonies to petri dishes containing only sterile malt agar. This was done in hope that spores projected from the colonies had adhered to the cover and would in turn be projected onto the sterile agar thus starting new colonies. Within 3 to 5 weeks new colonies began to form on the agar indicating that the spores had been projected. In some of the more active cultures spores were projected to the covers of the petri dishes and caught in drops of water formed by condensation. Many of these subsequently germinated in the liquid.

DEVELOPMENT OF CULTURES

Single point inoculations were made in the center of petri dishes containing approximately 15 cc. of malt agar. In 5 days new growth could readily be seen. Mature colonies developed within 3 to 4 weeks.

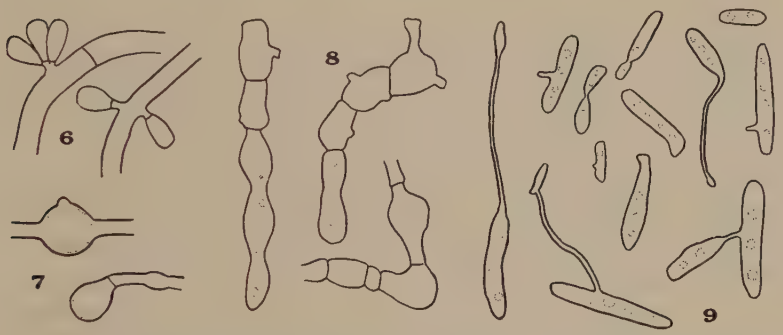
In about one out of every ten inoculations only a single giant colony 2.5–4 cm. in diameter developed (FIG. 1). In the majority of inoculations, however, other colonies were also formed. These were small colonies similar to the giant colonies 0.1–1 cm. in diameter (FIG. 2, surrounding giant colony) and gelatinous pustules 0.1–0.5 mm. in diameter (FIG. 3, to right of giant colony). Often these colonies coalesced to form one large colony by confluence. The small colonies were sometimes so numerous that they coalesced before becoming fully developed and formed a single mat-like colony that covered the entire surface of the agar. The gelatinous pustules were at first discoid to pulvinate and then became irregular.

These colonies produced three types of spores. One type was ellipsoid to obovoid, 4–5 × 1.8–2.5 μ, borne singly or in clusters of 2 or more on short sporophores (FIG. 6). These spores gave evidence of being forcibly projected from their sporophores; however, no mechanism for this type of spore discharge could be ascertained. These are probably the same type of structures that Brefeld reported developing from spore cultures in liquid media. He did not observe the spores being projected because all his cultures were made in liquid. The second type consisted of thick-walled chlamydospores, spherical to obovoid, 6–12 × 5–8 μ, which may be intercalary or terminal (FIG. 7). The third type consisted

FIGS. 1–5. Cultural forms of *Dacrymyces Ellisii*. 1. Giant colony, × ½. 2. Giant colony surrounded by small colonies, × ½. 3. Giant colony with gelatinous pustules at right, × ½. 4. *Tilletiopsis* colony, × ½. 5. *Tilletiopsis* colony, × 6.

of the elongate ballistospores described below. The hyphae in older cultures often formed oidia (FIG. 8).

Approximately 300 transfers were made during an 8-month period. All were from cultures originally made from either *6425* or *6426*. The only discernible difference between *6425* and *6426* is that the latter is usually slightly lighter in color. All precautions were taken during the handling of these cultures to assure the absence of any contamination. During this period the colonies



FIGS. 6–9. Spore forms of *Dacrymyces Ellisii*. Drawn with the aid of camera lucida, × 1000. 6. Spores of *6425* which are forcibly projected. 7. Chlamydospores. 8. Hyphae breaking up to form oidia. 9. Ballistospores budding and germinating by repetition.

already described always developed. However, on four separate occasions a third type of colony forming ballistospores developed from *6426* (FIGS. 4, 5). These colonies are white when young and later become tan. They are at first almost completely composed of spores. More hyphae form as the culture ages. The spores are elongate and slightly curved, 11–25 × 2–3 μ, averaging 17× 2.6 μ (FIG. 9). These spores germinate by budding and by repetition, and they are forcibly projected. Oidia will form from hyphae in the older cultures.

DISCUSSION

The forms which appear in the various cultures of *Dacrymyces Ellisii* exhibit a little known phase in the life cycle of that fungus. The most interesting aspect of the results obtained in this work was the appearance of spores which were projected from their sporophores and the occurrence of certain spores which germinated by

budding and by repetition. The white colonies which arose from *6426* have all the characteristics of the genus *Tilletiopsis*. It is believed that they are definitely members of this group. Furthermore, the projected spores found in the other colonies also show a resemblance to the family Sporobolomycetaceae.

The position of the Sporobolomycetaceae has always been questioned. There have been various opinions as to its correct taxonomic position. Buller (**2**) thought it to represent reduced forms of Basidiomycetes. Derx (**3**, **4**) has also stated that the group probably belongs in the Basidiomycetes. In speaking of the genus *Tilletiopsis* Nyland (**6**, **7**) states that the lack of parasitism alone is hardly sufficient reason for excluding that genus from the Ustilaginales. Olive (**8**) has expressed the opinion that the Sporobolomycetaceae may be related to the Tremellales. Martin (**5**) has included the Sporobolomycetaceae as an addendum to the Tremellales in his recent Revision of the North Central Tremellales. Others have placed these fungi in the false-yeast group.

The forms described in this paper show clearly that such fungi may represent part of the life cycle of *Dacrymyces Ellisii*. The life cycle of this species, therefore, would consist of the perfect stage characterized by the formation of basidia, an imperfect stage marked by the formation of conidia and an intermediate phase which takes the form of ballistospores which germinate by repetition and budding.

This constitutes further evidence that the Sporobolomycetaceae have a definite taxonomic relation with the Tremellales.

Studies are being made at the present time of cultural forms of other genera of the Tremellales.

This work was done under the supervision of Professor G. W. Martin.

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SOME VIEWPOINTS ON THE PHYLOGENY OF RUST FUNGI. I. CONIFEROUS RUSTS

E. E. Leppik

(with 5 figures)

Most recent mycologists (Dietel, Faull, Gäumann, etc.) agree with A. Möller's (**17**) proposal to derive the Uredinales or rust fungi from the order Auriculariales of the class Basidiomycetes. Their point of view rests mainly upon the marked similarity which exists in the structure of the phragmobasidium and the formation of basidiospores of the Auriculariales and Uredinales. There are two further hypotheses, that of de Bary (**1**) which derives the Uredinales from the Ascomycetes, and that of Brefeld (**2**) which connects them with the Zygomycetes. These hypotheses have not found further support since the question was thoroughly discussed by Gäumann (**4**, pp. 421, 586–592). But mycologists do not agree too well when it comes to the classification of this group of fungi. More than 4000 recent species of rusts are represented in such a variety of sporulating forms that it is difficult to subordinate all of them to the definite groups.

Up to the present time no definite phylogenetic system of Uredinales could be established. Gäumann (**5**, p. 586; **6**, p. 303) characterizes the present situation as follows: There is a complete anarchy in regard to the significance of all forms and types of life cycles of these fungi. How have they arisen, and how are they related? The answer to this question is difficult, for the Uredinales belong to the oldest known fungi and they have survived to our time, isolated as *"living fossils."* They have not, however, left us *petrified fossils* of their ancestors. Thus the answer cannot be given without considering at the same time the phylogeny of the Uredinales, their systematic division and the origin of their biological specialization.

Recently the history of rust fungi has been studied in connection with the phylogenetic and hologenetic development of their host plants, which method provides a new approach to the question of their phylogeny and classification. The present article attempts to summarize some results which have been reported in Germany and are not readily accessible to American mycologists.

THE PRESENT CLASSIFICATION OF RUST FUNGI

The present taxonomy of the rust fungi is based on the morphological structure of their teliospores. This method of classification, however, has been applied by various authors in different ways, and cannot be accepted without criticism (Leppik, **10**).

Although the teliospores may provide perfect characteristics for generic identification, their distinctive value cannot be used with the same effect for the classification of families and suborders.

During the long process of evolution the rust fungi have several times eliminated their former teliospores and have developed entirely new forms of spores in some other stage of the life cycle which have taken over the function of teliospores. Therefore, the teliospores of different phylogenetic groups may be similar but not always genetically identical organs. In most cases they are formed from the binucleate urediniomycelium. In *Coleopuccinia* they are derived from the aeciomycelium, in *Endophyllum* directly from aeciospores, in *Uredinopsis* from the intrasoral mycelium, etc.

Still more frequent are the cases where the teliospores in genetically related groups, responding to different ecological environments, have changed their shapes to unrecognizable forms. In these cases the teliospores may be genetically identical but are not similar morphologically. Because of their extreme plasticity the phylogenetic value of teliospores cannot be very great, and the term "teliospore" can be applied in a functional rather than in a genetic meaning. The question of origin and historical development of teliospores can therefore be approached with hologenetic viewpoints rather than phylogenetic, and will be discussed in a later chapter.

On the other hand, the stability of forms of basidiospores, urediniospores and pycnospores does not by any means furnish a better

basis for the phylogenetic classification of rust fungi than the diversity of teliospores.

THE EVALUATION OF AECIA FOR PHYLOGENETIC PURPOSES

The single-celled, binucleate aeciospores are typical of all heteroecious rusts. Their morphologic structure is stable and the slight variations in their dimensions and cell-wall structure have no special significance for phylogenetic distinction.

TABLE I

PRINCIPAL TENDENCIES IN THE DEVELOPMENT OF AECIA

Age of	Main types of aecia ← Progressive trend | Regressive trend →	Eras
Angiosperms	*Roestelia* → Aecioid, Protuberoid, Cornuoid, Roestelioid (subtypes); *Caeoma* II type: *Phragmidium*; *Aecium* II type: *Uromyces*	Neophytic
Gymnosperms	*Peridermium*; *Caeoma* I type: *Melampsora*; *Aecium* I type: *Uredinopsis*	Mesophytic
Ferns	Aecial stage on ferns unknown	Paleophytic

More valuable for phylogenetic purposes are the aecia or aecidia, the special sori where the aeciospores are formed. The principal tendencies in the development of aecia are sketched on TABLE I and can be compared with the main evolutionary trends of coniferous rusts, pictured on FIG. 1.

According to this table, the *aecium* I, type *Uredinopsis,* with well-developed pseudoperidium, could be one of the oldest in this group. It presumably arose as early as the end of the Paleozoic Era and reached stability in the Mesozoic on gymnosperms in a comparatively dry climate. This type delivered two early branches: *peridermium,* with an exceptionally thick pseudoperidium adapted to an especially dry environment, and *caeoma* I of *Melampsora,*

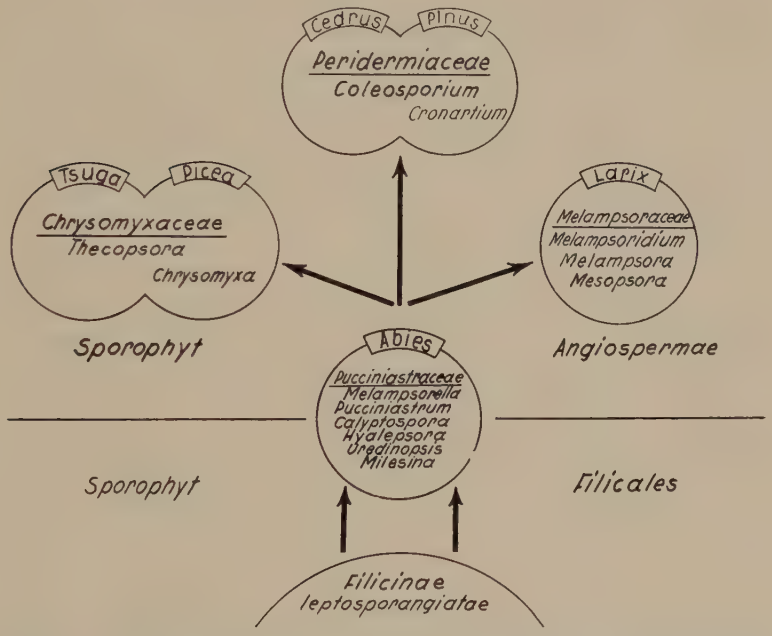


FIG. 1. Some main evolutionary trends of coniferous rusts on Abietaceae, as these can be established according to morphological structure and biological specialization of recent forms.

with a reduced pseudoperidium, adapted to a moist climate. Evidence is so far morphological, phylogenetic and hologenetic (Leppik **8, 10, 14**).

Later this type, without much change in its morphological structure, reached angiosperms, where it was distributed as the *aecium* II of *Uromyces* in the *Pucciniaceae,* the largest group of rust fungi.

In some cases the *Uromyces*-type could not adjust itself to special climatic conditions and was therefore replaced by *roestelia* and *caeoma* II.

Special attention should be given to the *roestelia* type of *Gymnosporangium*. The aecia of this type possess a modern mechanism, which regulates the distribution of spores in connection with changing weather conditions. This mechanism enables it to hold back the spore material in the sorus until the combination of temperature, humidity and wind velocity has reached a most favorable index for distribution. When this point is reached, a special mechanism empties the sorus and closes it again as long as new spores are ripening (Leppik **1949**).

This new achievement of a young rust family has proved effective and the mechanism has already been modified in several special cases. In fact *roestelia* has split itself into several subtypes or "typomeres": aecioid, protuberoid, cornuoid and roestelioid, which are provided with clear morphological characteristics (see TABLE I and Leppik **14**, from manuscript).

In this polymorphism and variability of *roestelia* subtypes is reflected the phylogenetic youthfulness of the genus *Gymnosporangium*. This is a new trend within the higher rust fungi, a mechanism to regulate the distribution of their spore material in connection with weather conditions.

In this way the arrangement of aecial types according to their historical development may provide a framework for the further phylogenetic considerations of the rust fungi.

AECIA AS INDICATORS OF CLIMATIC CONDITIONS

The aecia of rust fungi are intended to protect immature aeciospores against drought and to facilitate the distribution of these spores after their maturation. The protection of these spores against low temperature may be of some importance in localities where frost in the early spring is common. Several constant aecial types are, therefore, characteristic of certain weather conditions and may be evaluated as indicators of the climate. Except in some special cases, the aecia reflect the weather conditions of spring and early summer, which is the season of their appearance. A historical development of aecia may consequently reveal the climatic change of the past (Leppik **9**, **12**, **14**, **15**).

The general picture of a climate, as it is mirrored by different types of aecia, may be completed according to other sporulating

forms of rust fungi. Protected urediniospores point to a dry summer, whereas the development of a protective covering by the teliospores suggests a cold winter. To be sure, these suggestions can have practical value only if the origin of different types of rust sori can be connected with the historical development of their bearers.

The present-day distribution of types is not always decisive. The rusts with well-protected sori are also able to live in a moist and warm climate, although the opposite weather conditions are the essential prerequisites for their original development.

In the case of coniferous rusts, the comparative phylogenetic and hologenetic studies of these rusts in connection with their host plants enables us to restore the origin and historical development of some main types of aecia (see later). These studies also allow us to make some conclusions concerning the climatic conditions of the past, which have been responsible for the development and accomplishment of certain definite types of aecia.

The ancestral *prototype* of the aecia of coniferous rusts presumably had a tubular or cup-shaped, thick-walled pseudoperidium, which provided sufficient protection for the immature aecidiospores against drought in a moderately dry climate. This prototype can be assumed according to the morphological structure of numerous living representatives of coniferous rusts. Among the recent types, the *aecium* I of Pucciniastraceae comes closest to the ancestral prototype of the aecium.

The geographical distribution of recent fir forests in the Northern Hemisphere, the residence of *aecium* I on *Abies,* enables us to realize the climatic environment in which the early development of the aecial prototype has taken place. The moderately dry climate of fir forests with well established seasons, differs sharply from that of the tropical rain forests, the place where the ancestors of coniferous rusts presumably lived near the end of the Paleozoic Era. The emigration of rust fungi from the moist paleotropic climate into the temperate coniferous forests of the Northern Hemisphere was evidently the main cause of the rearrangement of their sporulating organs. All other forms of spores except the basidiospores received peridial covering, whereas the teliospores were embedded in the host tissue. Since that time, Urdineae sorosporae

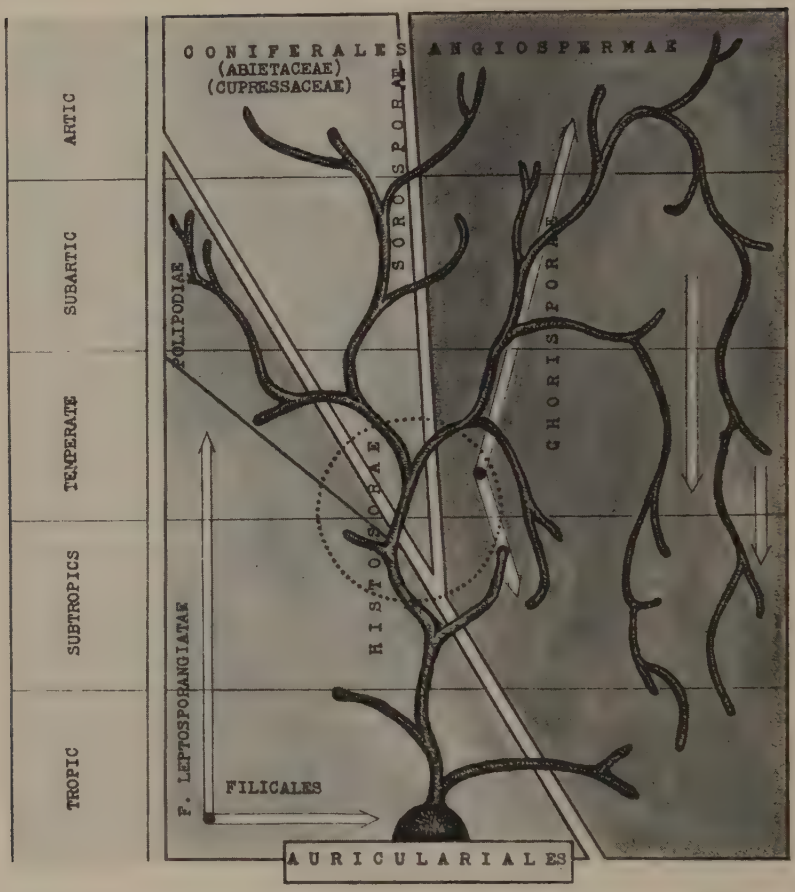


FIG. 2. An assumed genealogical tree of rust fungi, projected on the shaded fields of the host plants of these fungi. Originating from the tropical Auriculariales the main road of rust fungi leads from tropical ferns over conifers to the temperate and arctic angiosperms. White arrows indicate the main evolutionary trends of the host plants (see text).

became associated with the coniferous family Abietaceae and preserved this specialization to the present day: Their further evolution is closely connected with the historical development of Abietaceae. The splitting of the Abietaceae into the recent genera *Abies, Picea, Pinus, Larix* and *Cedrus*, which were adjusted to the various special climatic conditions, followed the splitting of the aecial prototype into the recent *aecium* I, *caeoma* I, and *peridermium*. All

these types represent the specific climatic condition in which they live.

In the morphological structure of *caeoma* I of *Melampsora* is reflected the damp climate of the temperate and subarctic forest regions of the northern hemisphere. A short, but moist spring with long days and short nights caused only a slight change of temperature between day and night. The moist soil covered with mosses, the retarded evaporation in the half-shadowed forests, and the moderate air currents are the further climatic prerequisites for the development of *caeoma* I. A flat caeoma without a pseudoperidium is able to produce more spores than an aecium, and is able to expose all of them to the air current at the same time. A tubular peridium would hinder this procedure. In the morphological structure of teliospores of *Melampsora* is further mirrored a long and hard winter of the northern hemisphere.

Larix, the main host of *caeoma* I, has responded to these special climatic conditions by changing its permanent needles to deciduous leaves, which is a rare phenomenon among the conifers. Other host plants of *Melampsora,* such as *Salix, Populus,* Orchidaceae, etc., that live in the same environment with *Larix* and *Melampsora,* and are exposed to the same ecological conditions, are all well-known mesophytes.

Peridermium, with several peridial layers, is one of the best protected aecia in the exceptionally dry climate of the pine forests. It can therefore thrive on *Ephedra* even in the half-desert areas of America and Asia.

THE RELATIONSHIPS BETWEEN THE DIFFERENT TYPES OF AECIA

There is much similarity in the morphological structure of aecia and aeciospores of coniferous rusts. The anatomy of the epispores, the formation of spores in chains, and the development of tubular pseudoperidia, all point to the common origin of *aecium* I, *caeoma* I and *peridermium.* They all inhabit closely related genera of the Abietaceae, which fact makes the mutual relationship of these rusts and the origin of their aecial sori from a common prototype still more conclusive.

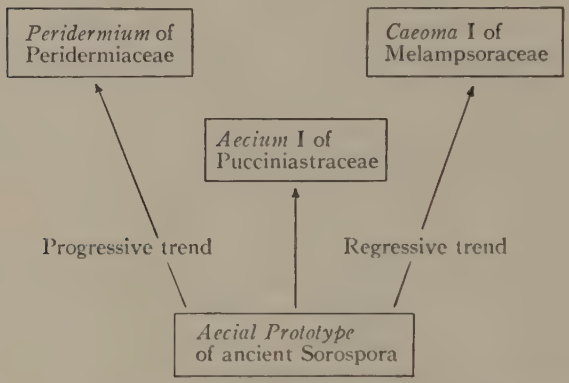
But none of the existing types can represent the aecial prototype, though *aecium* I of Pucciniastraceae is closer to it than any

other sorus. In all probability the present aecial types have evolved from a common prototype of ancestral Sorospora, as sketched in TABLE II.

There is an analogy in this scheme and in the evolutionary trends of coniferous rusts, as these are demonstrated on the recent groups

TABLE II

THE HISTORICAL DEVELOPMENT OF AECIUM I, CAEOMA I AND PERIDERMIUM FROM A COMMON PROTOTYPE



(see FIG. 3). The historical development of these main groups of Uredineae sorosporae also coincides with the evolution of the Abietaceae (see TABLE III).

Among the recent types, *aecium* I stays closest to the proto-aecium of Sorospora (see below). Concerning the development of the pseudoperidium, the *peridermium* is a progressive and *caeoma* I a regressive trend from this aecial prototype.

The *caeoma* II of *Phragmidium* evidently has no relationship with *caeoma* I and should be provided with another name.

The striking similarity in the morphological structure of the *aecium* I of *Uredinopsis* and the *aecium* II of *Uromyces* is very significant. Aeciospores of both groups have the same cell-wall structure, are formed in chains, and possess intercalary cells. Further evidence of the relationship between *aecium* I and *aecium* II can be obtained from hologenetic considerations.

The question of a genetic relationship between Pucciniastraceae (*aecium* I) and Pucciniaceae (*aecium* II) is one of the most fascinating problems, not only in the phylogeny of rust fungi, but

also in the origin of angiosperms, and should be discussed in another article.

The *roestelia* of *Eugymnosporangium* is a new sporulating form among the rust fungi. In spite of a marked difference in its morphological structure, this type has a clear phylogenetic and

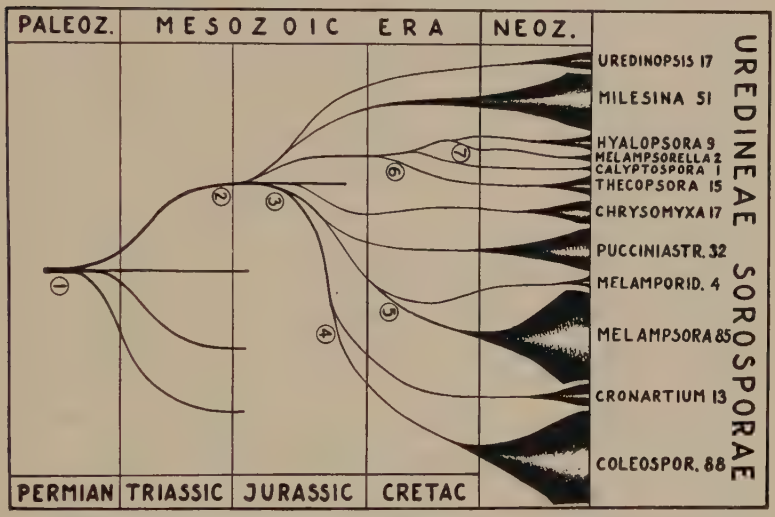


FIG. 3. Genealogic tree of Sorosporae. Encircled numbers are some points which may be connected with geological time table (see text).

hologenetic relationship with the aecium. Except some protuberoid forms, the main part of the subtypes of *roestelia* can be derived from the still living ancient cupulate sori of *Gymnotelium*.

RELATIONSHIPS AMONG THE DIFFERENT GROUPS OF CONIFEROUS RUSTS

The existence of a close relationship between different groups of coniferous rusts on the Abietaceae seems to be beyond doubt. The morphological structure of the aecia and aeciospores (see above), the coincidence of their biological specialization, and the geographical distribution of present forms makes the origin of these rusts from a common ancestral group highly probable. In spite of many controversies about the structure of their teliospores, most of the taxonomists at present are combining all coniferous rusts into a single group. But even the great variation of telio-

spores can now be interpreted as a result of several hologenetic tendencies within the same group.

In spite of a close relationship among the coniferous rusts none of the existing groups can represent the prototype for these rusts. All recent genera and families of these rusts have been, for many ages, parallel evolving groups, each without ancestral rights for the recent suborder of coniferous rusts.

The often used name "Melampsoraceae" for the coniferous rusts is rather confusing, and from the phylogenetic standpoint, quite untenable. The genus *Melampsora*, which is characterized by *caeoma* I, is a new, partly regressive trend, and cannot be considered as either a prototype or an ancestral group, for these rusts. Gäumann (6, p. 329) has already abandoned the old viewpoint and preserved the name "Melampsoraceae" only for a restricted group of coniferous rusts, the bearers of *caeoma* I. But every group of these rusts preserved some of the traits of their archaic ancestors. A comparative study of recent groups in connection with phylogenetic and hologenetic trends among these rusts and their hosts enables us to restore the morphological picture of their ancestors.

In a former article (Leppik 10) it was suggested that all families of coniferous rusts that had a prevalent tendency to close their main forms of spores into definite sori, be united into a suborder Sorosporae.

There is some heuristic value and also some practical use in providing the ancestral group of Sorosporae, the bearer of the aecial prototype, with a provisional name *Sorospora*. Its existence in the late Mesophytic is beyond doubt.

In view of the recent rich flora of the suborder Sorosporae, it is not difficult to establish the main morphological characteristics of the ancient *Sorospora*. Most typical of *Sorospora* is its formation of spores in specially protected sori. Except basidiospores, all other spores are covered with a pseudoperidium and are embedded in host tissue. Teliospores are, in the beginning of this new evolutionary trend of rust fungi, disorganized in the host tissue, but have the clear tendency toward the well-organized subcuticular sori. Urediniosori are also covered with a pseudoperidium.

Amphiospores may be assumed for *Sorospora,* because they are typical for some lower coniferous rusts, but are gradually disappearing in higher forms.

In this image of combined sporulating forms of *Sorospora* is reflected a picture of an archaic climate from the late Mesophytic Era. A moderately dry spring and summer are the necessary prerequisites for the evolutionary development of a protective pseudoperidium of aecio- and urediniosori. A moderately cold winter is required for the genesis of the different types of teliospores which are represented in the suborder Sorosporae. The variation of the teliospores further mirrors several subtypes of the winter climate of the past.

THE PLACE OF ORIGIN OF CONIFEROUS RUSTS

According to the available material and the above presented conclusions, some assumptions can be made about the place of origin of the coniferous rusts.

All main soral types of suborder Sorosporae reflect in their structure a temperate or subarctic climate, which weather conditions may also be assumed for the development of ancestral *Sorospora.*

The aecio- and uredosori are covered by a well-developed pseudoperidium, which protects the maturing spores against drought during the spring and summer, i.e. the seasons of their appearance. The morphological structure of the teliospores and the position of the teliosori in the host tissue mirror the different accommodations that have occurred to protect these spores against low temperatures in winter. The origin and development of a protective pseudoperidium in a moist climate, or of winter spores in the tropics, would be quite improbable. Neither is it possible to imagine the origin of the deeply rooted heteroecism of coniferous rusts in a region without climatic seasons.

The historical development and recent geographical distribution of Abietaceae exclusively in the Northern Hemisphere restricts the origin and living area of the coniferous rusts to this territory of their host plants.

Under these conditions it is rather discouraging to look for the place of origin of coniferous rusts in recent temperate Europe or

America, which provinces had a tropical climate up to the middle of the Cenozoic Era. *Sorospora* definitely could not arise in the paleotropic Europe or America, where the weather conditions have been quite unsuitable for this fungus until the late Tertiary Period. According to the paleontological records occasional climatic prerequisites existed in the recent arctic regions during the Jurassic and Cretaceous Periods.

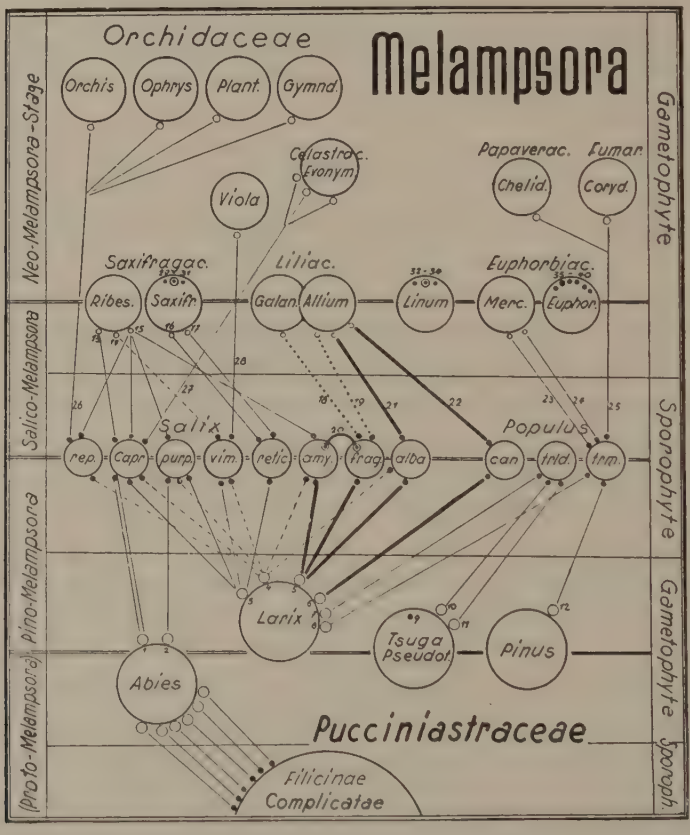


FIG. 4. The hologenetic ladder of biological specialization of *Melampsora*. According to this ladder the phylogenetically oldest species of the *Proto-Melampsora* stage form the aecia on *Abies* and teliospores on ferns. The next stage of *Pino-Melampsora* connects the Abietaceae with the Salicales. Further, the youngest species of the *Neo-Melampsora* stage step from the Salicales to the higher angiosperms. (From Leppik 12, 1938. For explanations see text.)

It is quite certain that the birthplace of ancestral *Sorospora* was in the fir-fern forests of *Proto-Abies* in the Northern Hemisphere. Both host plants, *Proto-Abies* and a young fern group of *Polypodiaceae,* adjusted themselves to live in the northern temperate provinces where they formed extensive fir forests, with ferns as the main undergrowth. This is clearly demonstrated in the biological specialization of Pucciniastraceae (see above). Other conifers of the Taxodiaceae and Araucariaceae could not live in the territory of fir forests. The invasion of coniferous trees toward the north was continued by the formation of spruce and hemlock forests, the habitat of *Chrysomyxa.*

Accompanied by *Melampsora,* the genus *Larix* could force its way still farther north to the moist subarctic regions. In the hologenetic ladder of *Melampsora* is mirrored the historical development of the tamarack forests. Into the mixed *Larix-Salix-Populus* forest enter gradually a number of angiosperms: *Saxifraga, Ribes, Allium, Calanthus, Evonymus, Viola,* different Orchidaceae, etc. (see FIG. 4).

After the cooling of the northern hemisphere during the Cenozoic Era, the coniferous forests shifted southward and reached their present location probably in the end of the Tertiary Period.

SOME VIEWPOINTS ON THE PHYLOGENY OF ABIETACEAE

No one today doubts either the close relationship between the conifers *Abies, Picea, Tsuga, Larix, Cedrus,* and *Pinus* or their origin from common ancestors. Taxonomists are combining all these genera into a single family Abietaceae, recognizing, with this name, the priority of *Abies* in the whole group. Less definite, and more misleading, is the formerly used family name Pinaceae. The genus *Pinus* cannot be considered as a type for the whole group, but is rather a specialized side line in the evolutionary trend of *Proto-Abies.*

The common origin of the Abietaceae from a single ancestral group is further demonstrated in the close relationship of coniferous rusts, which are deeply rooted in the main genera of these conifers (see above).

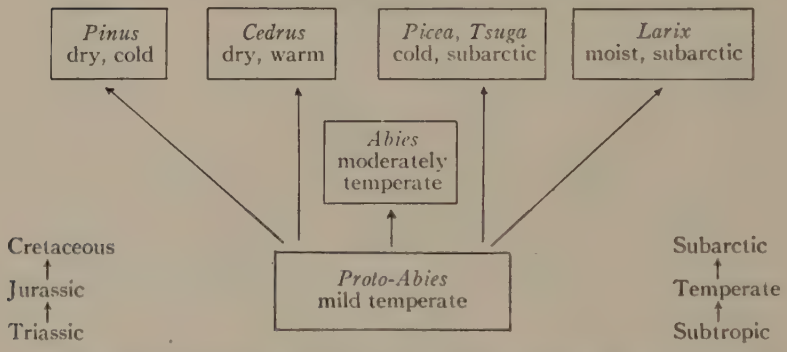
The phylogeny of the conifers has already been considered by many students and described according to the rich flora of their

fossils by E. Irmscher, R. Kräusel, W. Studt, W. Gothan, E. W. Berry, R. Florin and several other paleontologists. The author of this article studied the evolution of conifers in connection with the phylogeny of their rust fungi. Some preliminary reports about this work are published in several former papers (Leppik **8, 9, 10, 11, 12, 15**).

If the ancestral group of recent Abietaceae is provided with the provisional name *Proto-Abies,* the following preliminary sketch may be outlined according to the research records mentioned above.

TABLE III

HISTORICAL DEVELOPMENT OF ABIETACEAE



If we restrict the use of the name *Proto-Abies* only to a definite phylogenetical group, which gave rise to the recent family of Abietaceae, several concrete conclusions can be made about this ancestral group according to existing genera and their petrified remnants. Aside from their individual differences, all existing genera of Abietaceae have many common traits that they inherited from their ancestors, i.e. from *Proto-Abies*. All existing genera of Abietaceae inherited also from the *Proto-Abies* their rust fungi, a group of highly specialized parasites, which have accompanied their hosts since their early beginning. The phylogeny and biological specialization of the rust fungi therefore help to trace the historical pathway of conifers, and to establish the relationships between different groups of their host plants. The different soral types of the rust fungi indicate further the special climatic conditions that are responsible for their appearance (see above).

According to these considerations the place of origin of *Proto-Abies* could be located on the northern hemisphere where all recent Abietaceae still live. There is clear evidence that the historical development of Abietaceae was an evolutionary trend among the conifers from a subtropic area toward the boreal one. This trend is also reflected in the development of a protective covering in the sporulating forms of accompanying rust fungi (see above). Another trend was directed from moist soils toward the dry climate.

For *Proto-Abies* can therefore be assumed a mild temperate or subtropic area with well-established climatic seasons. This can be concluded from the well-protected sori and heteroecism of *Sorospora*. A further indication of the climatic seasons may be the annual rings of some Protoabietinae. That the annual rings are caused by growing seasons, interrupted by winter and not by drought, can be concluded from the well-protected winter spores of *Sorospora*.

According to these prerequisites, the equatorial tropic formed a climatic barrier toward the south, which neither *Proto-Abies* nor its phylogenetic successors could ever pass over, except in high mountain areas.

According to geological records the whole territory of recent temperate Asia, Europe, and America has had presumably during the whole Mesozoic Era a tropical climate. The area of ancient *Proto-Abies* can therefore be assumed to be still far north in the present arctics, where its first remnants may lie hidden under the ice and snow. The phylogeny of the rust fungi and of several other groups of plants and animals points in the same direction. Because of the severe weather conditions, hardened plants and trees returned from time to time to more southern provinces, where they may also appear in paleontological records. These fugitives from the arctic can now be traced according to their accompanying rust fungi, whose spore forms indicate their northern origin.

Another assumption about the place of origin of the recent fir family somewhere in the paleotropic mountains of Eurasia is less evident. Neither Abietaceae nor their rusts contain montane types; these are common to the Cupressaceae and *Gymnosporangium* (Leppik **9**, **14**).

To be sure, *Proto-Abies* has, in ancient times, had several related groups with which it has had common, but still earlier ancestors, probably from the group of Prototaxodiaceae. These intermediate forms between Abietaceae, Taxodiaceae, or Araucariaceae, respectively, are arranged by R. Kräusel and W. Eckhold to an indefinite group of Protoabietineae. Our *Proto-Abies* would be a definite phylogenetic group of Protoabietineae, containing the ancestors of recent Abietaceae.

Some of these groups of Protoabietinae could have been infected with *Sorospora*-related rusts. There has been found an aecium fossil on *Glyptostrobus*, which is very similar to our aecial prototype.

Some of the ancient Protoabietineae also reached the southern hemisphere. *Cupressinoxylon Kräuseli* Eckh. from Argentine in Jurassic, *Pityoxylon* Kurtz from Patagonia in Cretaceous, *Planoxylon Hectori* Stopes from New Zealand in Cretaceous, and an uncertain find from Australia by von Ettingshausen, are some examples. But all these ancient conifers lived outside of the *Proto-Abies* area, became extinct without further successors, and cannot therefore have any direct phylogenetical relationship with our recent *Abietaceae*.

The time when the *Proto-Abies* first appeared may be assumed to be in some part of the Triassic Period, from which fossils of Protoabietinae are found in several parts of the northern hemisphere.

There is a remarkable similarity in the phylogenetic development of Abietaceae and their rusts, which fact can be best seen according to the corresponding TABLES I, II, III and FIGS. 1, 2 in the text.

THE HISTORY OF CONIFEROUS RUSTS

The historical pathway of the rust fungi begins with the lowest forms on ferns, continues its course over gymnosperms to the lower angiosperms, and finally reaches its destination on the upper groups of angiosperms (see FIG. 2). On this long road, the conifers play an important role in connecting the lower rusts on ferns, and their highest forms on angiosperms (encircled area on FIG. 2). This main phylogenetic trend of rust fungi is clearly exposed in their biological specialization. It can also be traced back phylogenetically and holo-

genetically. Some of the oldest fern rusts still live on their paleozoic host groups and have also preserved their ancient forms of spores. On the contrary, the medieval rusts prefer to inhabit the gymnosperms; whereas the modern forms of these fungi are exclusively distributed on angiosperms. The relative phylogenetic age of

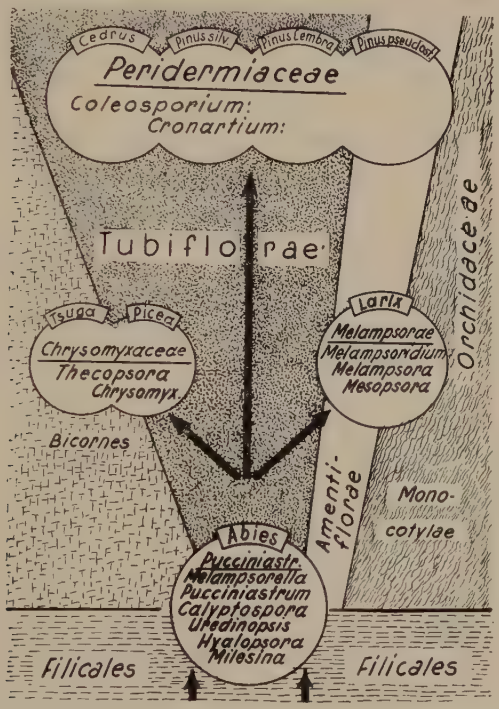


FIG. 5. The advancement of biological specialization of the coniferous rusts and their distribution among the different groups of angiosperms. In shaded sectors are pointed the phylogenetic groups of flowering plants which are inhabited by coniferous rusts.

certain groups of rust fungi can now be determined according to several hologenetic ladders, and some cases of unilateral pleophagia and their irreversible biological specialization (Leppik 8, 10, 12, 15).

The evolution of coniferous rusts is strictly dependent upon the historical development of their host plants. Every new group among the host plants provides a new opportunity for the rusts, and the evolution of the Abietaceae provides the pathway on which the coniferous rusts have to evolve. The comparative study of

the phylogeny of the conifers therefore helps to solve some of our problems (Leppik **11**).

The rich flora of petrified fossils among the Abietaceae enable one to connect the phylogeny of both conifers and their rusts with the geological time table. Uredineae sorosporae will probably be one of the first groups among the rust fungi whose phylogeny can be established with descriptive methods and whose history can be provided with some chronological dates (see FIG. 3).

On conifers, a number of old rust forms have survived which enable us to reveal the phylogenetic threads between some older groups of these fungi. It is very remarkable that on the fir trees of the genus *Abies* are found the *oldest* heteroecious rust forms, which still preserve their teliospores on ferns as *Milesia, Uredinopsis* and *Hyalopsora* (FIG. 1).

Other coniferous rusts: Chrysomyxaceae on related *Picea* and *Tsuga,* Peridermiaceae on *Pinus* and *Cedrus,* and Melampsoraceae on *Larix* all reveal characteristics of more advanced forms and evolve their teliospores on angiosperms.

This fact indicates that *Abies* should be considered as one of the phylogenetically oldest genera in its family, which viewpoint is, however, not in accordance with paleontological records. The petrified fossils of *Pinus* and *Picea* are known from the Triassic and Jurassic Periods, whereas these of *Abies* only since the Cretaceous. The late appearance of *Abies* in the paleontological records may be explained by the fact that fir cones are extremely fragile and do not produce petrifications. But to identify the fir-tree fossils only by wood remnants is very uncertain. Therefore, other indirect ways must be used to determine the phylogenetic history of the fir tree.

Another indication of the old age of the fir tree may be the unilateral pleophagia (polyphagia) between the rusts on genus *Abies* and numerous fern plants of family *Polypodiaceae.* By the irreversible development of biological specialization of rust fungi (Leppik **14**) it is impossible to imagine that so many rusts from numerous fern genera have joined together to form their aecia on *Abies.* More reliable is the opposite supposition that a group of rust fungi from *Abies* invaded many representatives of *Polypodiaceae,* in

which case the genus *Abies* must be considered phylogenetically older than the Polypodiaceae.

Some more indications for the phylogenetic development of coniferous rusts have been discussed in former articles (Leppik 10, 11, 12).

An attempt is made in FIG. 3 to sketch the phylogeny of the whole group of Uredineae sorosporae. In the Jurassic Period *Sorospora* was split into several new strains and groups. Some of these groups: Pucciniastreae and Chrysomyxeae preserve their main characteristics and their way of biological specialization. Other groups have a clear inclination towards different ecological conditions on specialized hosts.

Peridermiaceae (Coleosporieae and Cronartieae) on *Pinus* and *Cedrus* are specialized for extreme dry, and Melampsoraceae on *Larix*, for a moist climate. They lost their contact with ferns and have found new hosts for the teliospores, from different groups of angiosperms.

THE BIOLOGICAL SPECIALIZATION OF CONIFEROUS RUSTS

The study of biological specialization of rust fungi among some groups of host plants enables us to determine the period when these hosts first were infected by rusts during their phylogenetic development.

These periods of infection can be further determined by observing when the concerned host groups have split into new forms. If there are enough fossils for study, as in the case of Abietaceae, the periods of infection and the time of splitting into new forms can be connected with the geological time table and the relative age of studied groups can thus be determined.

These comparative studies can disclose most valuable facts about the phylogeny of both rust fungi and their host plants, and can provide some new viewpoints for scientific criticism.

Some points in the phylogenetic development of coniferous rusts which can be connected with a geological timetable (FIG. 3)

1. An ancestral group of blister rusts, which formed its aecia on a group of ancient conifers, can be assumed to have existed in Perm and Triassic about 240,000,000 years ago. Teliospores have presumably been formed on ferns.

2. *Sorospora* lived on ancestors of *Abies* (*Proto-Abies*), and ferns during the Triassic and Jurassic periods in the northern hemisphere (see above).
3. The splitting of *Sorospora* into new forms can be accounted for by the formation of new phylogenetic groups among the *Proto-Abies* in the Jurassic period, some 200,000,000 years ago. This point can be assumed only approximately according to fossils, which show well differentiated groups of Abietaceae since the beginning of the Cretaceous Period.
4. The separation of *Colcosporium* from Cronartiae can be more accurately dated chronologically to the Jurassic period, when *Pinus* was split into the subgenera *Haploxylon* and *Diploxylon*. *Cronartium* is evidently older; its numerous species inhabit both subgenera of *Pinus*. *Colcosporium* is restricted to the subgenus *Diploxylon,* but invades also *Cedrus*.
5. *Melampsora* and *Melampsoridium* have existed as independent groups since the splitting of the Betulaceae into *Betula, Populus* and *Salix* in the Cretaceous period, about 150,000,000 years ago.
6. The formation of *Thecopsora* occurred during the separation of *Picea* from *Abies* in Cretaceous.
7. The separation of *Melampsorella* from *Hyalopsora* occurred during the formation of Caryophyllaceae and Boraginaceae in Cretaceous.

THE HOLOGENESIS OF RUST FUNGI

All genetically related groups of organisms, species, genera, families, etc., no matter how heterogeneous they are or where they live, are united in a *phylum*. The genesis and historical development of a phylum is called *phylogeny;* it provides the essential foundation for taxonomy.

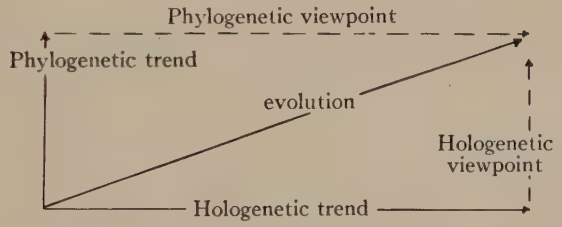
A phylum, however, cannot exist alone, because its life and evolution is dependent upon other groups and upon its environment. In fact, a number of species from different phyla, even from different kingdoms, live contemporaneously in the same environment, forming certain associations, communities, biocoenoses or other combinations. No matter what their phylogenetic origin is, nor where they come from, they have to live together and adjust themselves to each other.

Since the early beginning of the organismic world, animals have been dependent upon plants for their source of food. But plant life is conceivable only by the coexistence of animals, who prepare the food for the plants and serve other essential functions such as pollination, seed distribution, etc. Most phyla of organisms are not only biotically interdependent, but such interdependence is an evolutionary causative agent of prime importance. The inter-

relationships of organisms really force them to live and evolve together as a *biotic whole* or *holon.*

According to a proposition of F. Merkenschlager, the genesis and historical development of the biotic whole may be called *hologeny*. It provides the essential basis for *typology* (Leppik **15**).

Considered in this way, the historical development of every organism is governed by two different groups of factors: *phylogenetic* and *hologenetic*. Both act independently of each other and are attempting to enforce the evolution of the organism into different directions, as follows:



The evolution of the organism really is the resultant of these two components (Leppik **15**).

In our case the hologenetic trend of the rust fungi is dependent upon the evolution of their hosts. Their existence is impossible without host plants and the evolutionary pathway of their hosts is compulsory for the rusts.

Phylogenetically, rust fungi show remarkable stability. Their phylogenetic characteristics, for example basidiospores, are therefore very constant. However, they have to follow their fast-evolving host plants and adjust themselves to different climatic conditions, from the extreme tropic to the extreme arctic. This need for permanent change makes rust fungi hologenetically very plastic. They have to form new types of spores and adjust their sori to different climatic conditions.

Descriptive hologeny is mainly concerned with the study of the principle of interrelationships between main groups of phyla, i.e. the interdependence which binds organisms inseparably to each other. These relationships are: saprophytism and parasitism,

symbiotism and antibiotism, synagonism [1] and antagonism, mutualism and commensalism, etc. In many cases the organisms are dependent upon each other simply as producers and consumers. Analogically with the relation between phylogeny and taxonomy, hologenetic studies provide the foundation for *organismic typology* (**16**).

THE PHYLOGENETIC CHARACTERISTICS OF RUST FUNGI

The most typical phylogenetic characteristics of the whole order of rust fungi are their peculiar way of forming the basidium, bearing single-celled basidiospores, and a constant alternation of generations. These characteristics are constant for the whole order and do not change much during the evolution of these fungi. Their history is long, presumably covering more than 300,000,000 years.

The tender basidia and basidiospores of rust fungi presumably were formed in the tropics during the carboniferous period of the Paleozoic Era. Their formation demands a moist tropical climate with a high temperature. According to Gäumann (**5**, p. 546), the basidia of *Jola javensis*, from the related order of *Auriculariales*, are formed in the damp mountain forests of West Java with an optimal air temperature of 18° to 26° C. and a relative humidity of not less than 90 per cent. These conditions prevail in the rainy season on the moss carpet between the undergrowth, in the late morning to afternoon. After an hour in free air, with a relative humidity of 70 per cent or a temperature below 15° C., the fructification stops.

The rust fungi preserve their tender basidiospores and their tropical way of formation even after the long journey from the tropics to the arctic. The relative moisture and temperature, necessary for the formation of basidiospores in the arctic, differs only by some few degrees from that of the tropics. To survive in the arctic, the rust fungi had to develop several new forms of spores and sori, some with very thick cell-walls. But presumably every arctic rust can survive, where its host turns back to the tropics. These secondary tropic rusts are highly confusing to the taxono-

[1] *Synagonism* (opposite to antagonism) is a special case of mutualism, a symbiotism where the partners are not bound bodily (Leppik **15**, p. 11).

mist, but offer an excellent opportunity to trace the phylogenetic pathway of both the rusts and their host.

THE HOLOGENETIC CHARACTERISTICS OF RUST FUNGI

One of the most plastic spore forms is the teliospore of rust fungi. In a cold climate they have to fill the ecological task of hibernation and have to adjust themselves to extreme conditions. The evolutionary trend of teliospores can therefore not be directed only by inside phylogenetic forces, but is governed by outside hologenetic factors. By their restricted phylogenetic value the teliospores deliver perfect characteristics for tracing the hologenetic pathway of rust fungi. In smaller groups the hologenetic characteristics of teliospores are fixed phylogenetically and can be used for identification of genera.

Aeciospores are characteristic of the heteroecious rust fungi of the colder and drier climate. The single-celled aeciospores are stable, but their sori are adjusted to protect immature spores against drought. They provide very good characteristics for judging climatic conditions and hologenetic tendencies of the rust fungi.

THE IRREVERSIBILITY OF BIOLOGICAL SPECIALIZATION

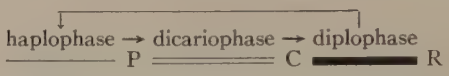
A new rust form adjusts itself regularly to some new group of host plants, which is phylogenetically younger than the host plants of its origin.

This rule was first observed by W. Tranzschel and confirmed by P. Dietel and Leppik (**14**). Both workers, Tranzschel and Dietel, used this rule to predict the unknown partners for the life cycle of heteroecious rusts. In fact they could establish a number of aecidial hosts and add greatly to our knowledge of the life cycles of several rust fungi.

From Tranzschel's rule can be concluded the irreversibility of the biological specialization of rust fungi (Leppik **14**). When a new fungus form is adjusted to a new group of host plants, its successive generations do not return to their old host plants. This rule is most valuable for the phylogenetic studies of both rust fungi and their hosts. In the heteroecious rust fungi, the irreversibility of specialization leads to the peculiar "hologenetical ladder," as

was first demonstrated by *Melampsora* (Leppik 12), and then by *Gymnosporangium* (Leppik 9, 14) and is also evident in Pucciniaceae (Leppik 8).

The evolution of the rust fungi is closely connected with an exactly fixed irreversible alternation of generations, as follows:



Each of these phases ends with a cytological change of nuclei: plasmogamy (P), cariogamy (C) or division of reduction (R). In *autoecious* rusts, both the haploid (gametophyte) and dicarioid (sporophyte) thalli live on the same host plant; in *heteroecious* forms each generation has a different host (see Gäumann 8).

TABLE IV

HOLOGENETIC LADDER OF MELAMPSORA (AFTER LEPPIK, 1948)

Hologen. phases of *Melampsora*	Evolution of biological specialization of *Melampsora*	Alternation of generations
Auto-Melampsora	● Teliospores on angiosperms	Sporophyte
Neo-Melampsora	○ Aeciospores on angiosperms	Gametophyte
Salico-Melampsora	● Teliospores on Salicales	Sporophyte
Pino-Melampsora	○ Aeciospores on Pinaceae	Gametophyte
Proto-Melampsora	● Teliospores on ferns	Sporophyte

By regular evolution the heteroecious rust fungus cannot change both its hosts at the same time. One generation must keep its old group of hosts until the other generation is well established to a new host. Then the first generation frees itself from the old hosts and chooses some phylogenetically younger group of plants for its new host.

In this way, the rust fungus with its two generations, sporophyte and gametophyte, climbs the hologenetic ladder of evolution step by step. A sketch of a hologenetic ladder of *Melampsora* is made in TABLE IV and FIG. 4.

It is possible to connect the single steps of a hologenetic ladder with a geological time table and reconstruct a hologenetic time table for certain groups of rusts. Several ladders of different rust

groups may be united to form a permanent hologenetic system (Leppik 12).

Using hologenetic ladders of different rust groups in connection with the phylogeny of their host plants, it is possible to construct a schedule of the historical development of a whole group.

FIG. 4, small circles (○) refer to aecia, black dots (●) to teliospores. The dots within circles (⊙) point to rusts with both aecia and teliospores on the same host plant.

Some subgeneric groups of *Melampsora* are marked with solid and broken lines and may be characterized as follows:

——— = Urediniospores rounded, teliospores subepidermal.

- - - - - - - = Urediniospores rounded, teliospores subcuticular.

▬▬▬ = Urediniospores elongated, teliospores subepidermal.

..... = Urediniospores elongated, teliospores subcuticular.

The studied species of *Melampsora* are marked with numbers as follows: 1. *abieti-caprearum* Tub., 2. *arctica* Rostr., 3. *larici-epitea* Kleb., 4. *larici-caprearum* Kleb., 5. *larici-pentandrae* Kleb., 6. *larici-populina* Kleb., 7. *medusae* Thuem., 8. *larici-tremulae* Kleb., 9. *farlowii* Arth., 10. *albertensis* Arth., 11. *medusae* Thuem., 12. *pinitorqua* Rostr., 13. *ribesii-epitea* Kleb., 14. *ribesii-viminalis* Kleb., 15. *ribesii-purpurea* Kleb., 19. *allii-fragilis* Kleb., 20. *amygdalinae* Kleb., 21. *allii-salicis albae* Kleb., 22. *allii-populina* Kleb., 23. *rostrupi* Wagn., 24. *pulcherrima* (Bubak) Maire, 25. *magnusiana* Wagn., 26. *orchidi-repentis* (Plowr.) Kleb., 27. *evonymi-caprearum* Kleb., 28. *lapponum* Lindf. 29. *hirculi* Lindr., 30. *vernalis* Niessl, 31. *alpina* Juel., 32. *cathartici* Buchheim, 33. *lini* (Ehrh.) Lév., 34. *liniperda* Palm, 35. *euphorbiae-dulcis* Otth., 36. *euphorbiae* (Schum.) Cast., 37. *euphorbiae-gerardiana* W. Mueller, 38. *euphorbiae-silvaticae* (DC.) 39. *helioscopiae* (Pers.). This list is incomplete.

FIG. 4 shows the further advancement of biological specialization of the coniferous rusts and their distribution among the different groups of angiosperms.

SOME CONCLUSIONS

The idea of studying the phylogeny of the rust fungi in connection with their host plants is by no means a new one. W. Tranzschel (1918), A. Mordwilko (1922) and P. Dietel (1936) took some of the first steps in this direction and a number of other workers in biology have pointed to the great value and necessity of such studies. The main obstacle that hindered this work was the absence of a definite phylogenetic basis and the lack of an applicable method for these studies. The problem that arose was how to combine and coordinate the phylogeny of two such genetically dis-

tant groups as the rust fungi and the higher plants. The absence of fossils makes the restoration of the past history of the rust fungi extremely difficult.

A new way has been found to study the phylogeny according to *"living fossils"* (Gäumann), which are abundant among the rust fungi. The rusts are, in fact, a very special group among plants, and have preserved their ancient traits beside those newly acquired characteristics of modern types.

The *hologenetic studies* help to establish the relative phylogenetic age of different interrelated groups of organisms, even in cases of distant phylogenetic relationships. The *irreversibility of biological specialization* of rust fungi (Tranzschel, Leppik) enables us to establish the *hologenetic ladders* (Leppik), which reflect the real evolutionary trends and progress of the groups they represent. The ladders of different groups may be united to form a permanent hologenetic system which can be connected with a geological time table. In several cases of *unilateral pleophagia* (Leppik 11) it is also possible to determine the relative phylogenetic age of different groups of host plants which the rusts inhabit. In this way it is possible to trace the very obscure history of the rusts and to reconstruct main outlines to the phylogeny of one of the most difficult and diversified groups of fungi. The method is simple and easily applicable, whereas the results can be controlled and improved at any time according to living specimens.

The hologenetic method of consideration is not based upon presumptions or hypothesis, but is a very reliable way of describing objects and facts exposed in nature.

Both phylogeny and hologeny have the same aim: to picture the historical development of organisms according to present forms, or using for this purpose petrified remnants. But both these trends have different viewpoints.

Phylogeny studies the historical development of a certain genetically related *taxonomic group,* from the phyletic standpoint of view. *Hologeny* traces the development of interrelations of genetically not related *typological units,* from the holetic viewpoint of the biotic whole or holon.

The perception of these two disciplines offers two different methods in the contemplation of the historical development of

organisms that lived in the past and cannot be seen or described directly.

The accuracy and completeness of our picture of the actual course of evolution is enhanced when we view it hologenetically as well as phylogenetically.

To be sure, only some few groups of rust fungi have been studied up to this time from the hologenetic viewpoint. The whole picture is still incomplete and the evolutionary schemes as they are presented in this article need corrections as soon as the other rust groups are studied.

The purpose of this article is to present some results for further criticism and to win more workers to this new field.

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SHORT-SEASON ADAPTATIONS IN THE RUST FUNGI [1]

D. B. O. Savile [2]

(WITH 3 FIGURES)

Intensive botanical work in northern Canada in recent years, particularly by members of this Division, has led to an increase in our knowledge of the parasitic fungi in the arctic and subarctic regions. Each year since 1948, specimens of rusts have been obtained both through intentional collecting and through the scrutiny of all northern phanerogamic specimens acquired by the Division. Although such specimens are of great value in adding to our knowledge of distribution and host range, they inevitably lack much of the data that would enable a mycologist to understand the biology of the species concerned. This fact was forcibly impressed upon me upon examining the rusts secured from four northern areas in 1948. I was, therefore, glad of the privilege to serve during the three succeeding summers as botanist with Northern Insect Survey parties at Great Whale River, Que., on the southeast coast of Hudson Bay, at Chesterfield Inlet, Keew., on the northwest shores of Hudson Bay, and at St. Anthony near the northern tip of Newfoundland.

Great Whale River is a tree-line station with typical arctic tundra along the coast but a rapid transition into spruce forest in valleys a few miles inland.

Chesterfield Inlet is a truly arctic station, although about 220 miles outside the arctic circle. It is about 300 miles beyond the accepted tree line, and probably nearly 200 miles from the last outlying clumps of spruce. The area consists of severely glaciated granite gneiss with a maximum altitude of less than 100 feet for several miles inland, in a bleak, wind-swept region with a very

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severe climate. Southern elements in both flora and fauna are fully as scarce as at some stations much further north; several ferns are absent that occur abundantly in more rugged terrain some 300 miles northeast in the Melville Peninsula; and many plants are extremely depauperate.

St. Anthony lies in the part of the northern peninsula of Newfoundland that is north of Hare Bay. All this area is within seven miles of the sea. It is in consequence wind-swept and the trees do not attain great size; but it enjoys a much longer frost-free season than Great Whale River. Perhaps because of these circumstances the flora shows a curious mixing of southern and high arctic elements. *Abies balsamea,* which is absent from Great Whale River, is abundant, as are several weeds that have been introduced at Great Whale River but have failed to become established.

These three stations may fairly be taken to represent tree line, the arctic and the northern transitional forest respectively. The latter is in some respects a mosaic of tundra and boreal forest, rather than a true vegetation zone; but the transition is in many places of great depth and the forest usually poorly developed and not wholly typical of the boreal zone. Its recognition accordingly seems desirable. From field observations at these stations it has been possible to determine the approximate northern limits for rusts of different types and to study some of the adaptations that permit various species to persist despite a short season. Rusts with such adaptations are by no means confined to the north, but their predominance there is overwhelming. The adaptations are sufficiently varied and striking to merit consideration in some detail.

The distribution data of some species used as examples are naturally considerably at variance with those given in existing floras. Details of distribution will be given in later taxonomic papers.

PUCCINIACEAE

In temperate regions a substantial proportion of the rust fungi have a life cycle nearly or exactly like that of a heteroecious, long-cycled *Puccinia,* shown diagrammatically in FIG. 1. In the spring

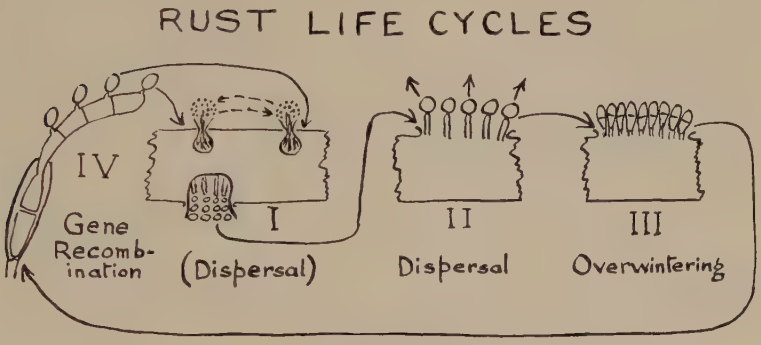

Fig 1.— Heteroecious, self-sterile, long-cycled Puccinia

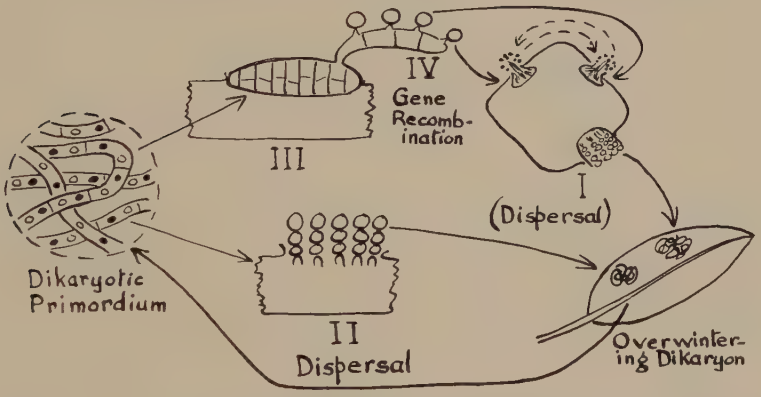

Fig. 2.— Typical Chrysomyxa with telescoped life cycle

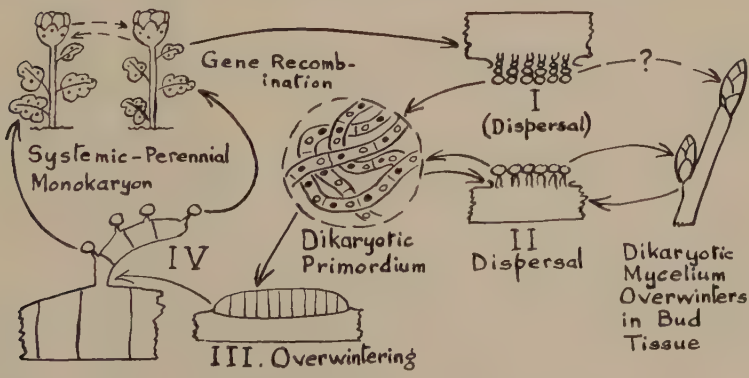

Fig. 3.— Variable life cycle in Melampsora epitea

the overwintered telia germinate, the basidiospores produce pycnia on the alternate host, the dikaryon is re-established and the aecia are formed. These stages function mainly to effect gene recombination, but the aecia have a secondary function in aiding dispersal. The urediniospores are conidia, serving to disperse and propagate the rust.

In southern Canada and the northern United States there is time for several uredinial generations; consequently the aecial and telial hosts may be separated by a considerable distance, and the association between them is not always obvious. As we go farther north the time available for uredinial generations is progressively decreased, and lower prevailing temperatures greatly slow down the rate of development. The decreased opportunity for build-up of the population makes survival impossible unless the alternate hosts are very close together. A number of grass and sedge rusts occurred at St. Anthony, but their spread was usually limited to two to six feet from the aecia and there appeared to have been an average of only about one full generation of uredinia before the formation of telia. At Great Whale River several *Carex* rusts occurred, but generally there was only a partial uredinial generation overlapping the formation of telia, and infection was usually limited to within a foot or so of the aecia. The only obligately heteroecious grass rust at Great Whale River was *Puccinia praegracilis* var. *connersii*. As has already been indicated (5, 6) the few uredinia and telia formed were almost contiguous with the aecia. It thus appears that tree line is about the limit for heteroecious, long-cycled species. It is probable that in a succession of favorable years such a rust may invade the barrens for a hundred miles or more, only to be forced back by the first poor season.

An example of an unsuccessful invasion of the barrens was noted at Chesterfield Inlet in 1950. A scattering of aecia was found on the leaves of *Ranunculus pallasii* in a shallow pond, but only on plants contiguous with the aquatic grass *Colpodium fulvum*. The rust is suspected to be a strain of *Puccinia rubigo-vera* specialized upon these two plants of similar habitat, and its normal range will probably prove eventually to be in the northern transitional zone, much of which is still botanically *terra incognita*. In

1949, a favorable season in much of the arctic and subarctic, uredinia were probably formed in quantity and enough reached Chesterfield Inlet to give a trace infection of telia. In 1950 the spring was late and on 9 August the aecia were just starting to open; most were open by 19 August, but by then the growing season was coming to a close. Growth of all plants seemed to have stopped by the end of the month and the *Colpodium* plants were ripening without any sign of infection. No further observations could be made, but it seems inconceivable that any telia could have matured under the cool conditions with frequent frosts that prevail in September.

The virtual suppression of uredinia as tree line is reached is not an adaptation, of course, but an inevitable and serious consequence of the environment. However, one characteristic is shared by *Puccinia praegracilis* and the unnamed *Aecidium* that may be interpreted as a short-season adaptation. In both these rusts there is a complete lack of pycnia. Presumably both species are self-fertile. The loss of pycnia actually represents more than the attainment of self-fertility; it is tantamount to the loss of all chance of cross-fertilization. This serious sacrifice could probably be borne only in well-adapted, successful species. It is likely to occur most frequently where it has some compensating value. In these northern species it has a clear advantage in reducing the time needed for completion of the life cycle. *Puccinia sorghi,* for example, needs about five days to mature the first aeciospores after mixing of the pycnial nectar, in the laboratory at 71° F. In the field, at about 55° F, it would probably require about ten days; and at tree line, with mean temperatures of about 45–50° F, the requirement might be about 15 days. Fifteen days saved out of a growing season of about three months may allow some spread by urediniospores when it would otherwise be impossible.

There are various other modifications of the heteroecious Puccinia type of life cycle, which, although also found in temperate zones, have evident advantages in the north. Several heteroecious rusts live over in the uredinial state on plants whose leaves remain green throughout the winter, obviating the need for proximity of the aecial host. *Puccinia poae-sudeticae* is sufficiently abundant on *Poa* spp. and *Trisetum spicatum* at various locations near tree

line to justify the belief that it may spread some distance into the barrens. *P. bistortae* overwintered at St. Anthony mainly as telia, producing aecia abundantly on *Coniosclinum chinense* and sparingly on *Ligusticum scothicum;* whereas Great Whale River it overwintered abundantly as uredinia, and no aecia were seen on the plentiful *L. scothicum.* This rust is common on *Polygonum viviparum* far into the arctic in areas where no aecial hosts occur. Possibly the abrupt ending of the growing season in the north has a physiological effect on the leaves that is conducive to continued production of uredinia, whereas in the south a gradual senescence promotes the change to telia. Other species in this category are *P. caricis,* which persists on certain *Carex* spp. near tree line, and *P. obscura,* which was recently found on overwintered *Luzula* leaves from Nome, Alaska. This process is not short-cycling as the term is properly understood, but is a sort of optional short-circuiting of the cycle. It is interesting to note that this is essentially the same device as that which permits cereal rusts to persist in warm climates where teliospores cannot germinate.

The autoecious full-cycled rust presumably has a slight advantage over its heteroecious counterpart, since a higher proportion of its basidiospores and aeciospores may be expected to fall on the appropriate plant. One example is *Phragmidium arcticum,* which was abundant enough on *Rubus acaulis* at Great Whale River to suggest that it might survive considerably farther north; a second is *Uromyces armeriae,* which was heavy in two colonies at the same station. Closely related to the last type are the autoecious brachycyclic species, best represented by *Puccinia hieracii,* which was plentiful at Chesterfield Inlet on *Taraxacum pseudonorvegicum.*

A further advantage may be confered by the systemic habit, as is shown by the occurrence of *Uromyces lapponicus,* with systemic-perennial aecia but localized telia, on *Oxytropis* spp. near or beyond tree line at various stations. The initiation of systemic infection appears always to require quite exacting conditions, but in this species the disadvantage seems to be offset by the prompt production of masses of aeciospores and a guaranteed survival even in years of very little plant growth. In point of fact telia of *U. lapponicus* were surprisingly scarce at St. Anthony and none could be found at

Chesterfield Inlet; and it is suspected that beyond tree line they often fail to develop.

Finally, the arctic and subarctic yield a number of short-cycled species of *Puccinia* and *Uromyces*, many of them widespread and abundant. These may be of the firm-pedicelled Eupuccinia type, in which the teliospores must germinate *in situ*, or of the Bullaria type with fragile pedicels, in which the spores may germinate when detached. The latter type enjoys some advantage over the former, because the teliospores may assist in dispersion. In each of these types the mycelium may be localized or systemic. The firm-pedicelled, systemic species are represented by *Puccinia holboellii*, which is fairly abundant on *Arabis* and *Draba* north at least to tree line. The firm-pedicelled, localized species are represented by *P. pulsatillae*, which occurs appreciably beyond tree line, *P. arenariae*, which occurs frequently far into the arctic, and *P. grindeliae* and *P. millefolii*, which extend at least to tree line. *P. drabae*, which is relatively common at Chesterfield Inlet and has been received from other arctic stations, and *Uromyces phacae-frigidae*, which is known from Alaska and Yukon, are examples of the systemic Bullaria forms. The best represented microcyclic forms are the localized Bullarias, several of which occur far beyond tree line. Examples are: *Puccinia blyttiana* (*P. ranunculi*) on *Ranunculus pedatifidus; P. heucherae, P. pazschkei* and *P. saxifragae-tricuspidatae* on *Saxifraga* spp.; *P. eutremae* and *P. oudemansii* on Cruciferae; and *P. mertensiae* on *Mertensia maritima*. All the short-cycled species listed lack pycnia and are presumably self-fertile. The majority attack rosette-forming or prostrate perennials (annuals are almost absent from the arctic), in which the leaves largely remain attached until the following season. This provision guarantees that the new season's leaves and flower stalks develop in close proximity with the sori of the previous year. In the arctic none of these species seems to produce more than one generation of spores in the year, although some, such as *P. arenariae* and *P. heucherae*, are freely leptosporic farther south. Here we have the ultimate simplification of the life cycle, with persistence assured by the close proximity of the new host tissues to the source of inoculum, but little opportunity for extensive spread. It is easy to understand, therefore, why in-

fection is often very heavy in a single clump of a host plant but absent from clumps a few feet away.

The preponderance of short-cycled rusts in mountain areas has been discussed by Arthur *et al.* (2), but the attempt by the same authors to show north to south distribution of micro- and macrocyclic species in North America was less successful, partly because the zones were too broadly delimited and partly because arctic and subarctic data were largely lacking. When data for the arctic are essentialy complete the microcyclic species will be found to outnumber the macrocyclic substantially; and such macrocyclic species as do occur will almost all prove to have some auxilliary means of overwintering.

CHRYSOMYXA

The genus *Chrysomyxa* provides interesting examples of short-season adaptations, as has already been indicated (4). FIG. 2 shows the life history of a typical, long-cycled species such as *C. ledicola*. All the stages found in a long-cycled *Puccinia* are adhered to, but the times of year for their formation differ considerably. These differences are possible because the telial host is always a plant with evergreen foliage. The rust overwinters on this host as a dikaryotic mycelium rather than as a telium. In the spring renewed growth gives rise to telia and uredinia nearly simultaneously. Usually the telia slightly precede the uredinia, but a single sorus sometimes produces both spore forms simultaneously. By the time that the sori are mature the new leaves of both *Picea* and the telial host are emerging from the winter buds; and the former become infected by the basidiospores, the latter by the urediniospores. Thus the processes of gene recombination and of dispersal and multiplication proceed simultaneously, telescoping the life cycle. In the south there may be additional spread by secondary uredinia, but in the north no uredinia are seen on the new leaves. The infecting aeciospores and urediniospores simply produce overwintering mycelia. Two representatives of this group, *C. empetri* and *C. ledicola,* persist in the dikaryotic state several hundred miles beyond tree line.

There are several modifications of the typical *Chrysomyxa* life cycle. *C. pyrolae* is systemic and full-cycled. The aecial stage is

systemic, but not perennial, in cones of *Picea,* and the dikaryotic mycelium is systemic and perennial in *Pyrola* and *Moneses.* The perennial mycelium assures the persistence of the rust, but the critical conditions for infection limit its spread. Aecia are seldom abundant and the species is scarcer than several others in most of its range. It is boreal to subarctic in distribution. *C. woronini* has a similar life history except that it has dropped the uredinial stage. The aecial stage is systemic in the young shoots of *Picea* and the dikaryotic mycelium forms perennial witches' brooms on *Ledum groenlandicum* and *L. palustre.* The only North American records are from near Dawson, Yukon, Great Whale River, Que., and St. Anthony, Nfld. It was relatively common at St. Anthony, but scarce at Great Whale River. *C. woronini* seems to be primarily a species of the northern transitional forest, but it is unlikely to prove common anywhere but in humid areas. Finally we have the microcyclic species, represented in North America by *C. weirii* on *Picea* and *C. arctostaphyli* on *Arctostaphylos uva-ursi.* The latter is limited to the boreal zone by the distribution of its host. *C. weirii* may eventually be recorded near tree line, but all records to date have been relatively southern. We must conclude that in *Chrysomyxa* the typical, full-cycled species can cope as well with a short season as any modified type that has yet arisen.

HYALOPSORA ASPIDIOTUS

Another mechanism that achieves the simultaneous, or at least overlapping, production of the different stages is seen in *Hyalopsora aspidiotus.* This rust produces amphispores, which germinate in spring, giving rise to uredinia and telia more or less simultaneously on the fronds of *Dryopteris disjuncta* in early or midsummer. Although the teliospores germinate immediately, the aecia do not develop on *Abies* until the next season or next but one; but they finally open in midsummer. Thus the cycle is so telescoped that all spore stages occur more or less simultaneously, yet without sacrificing genetic plasticity. Although *D. disjuncta* is not a dominant fern at St. Anthony, several colonies of *H. aspidiotus* were found. Aecia and uredinia sporulated freely in late July. I feel

confident that this species will eventually be found north to the limit of *Abies balsamea* (about Hamilton Inlet, Labr.), and it may well occur still further north in the uredinial state.

MELAMPSORA EPITEA

Our last and, perhaps, most remarkable examples of short-season adaptations are to be found in the willow rusts of the genus *Melampsora*. This group is a great complex of intergrading forms, a detailed taxonomic treatment of which must be postponed for some time. As far as the study has progressed it appears impossible to follow Arthur's (1) segregation of the complex into four species. The disposition even of many southern collections is difficult; but the numerous northern forms completely obliterate the presumed distinctions, and the situation is complicated by the fact that in the north aecia are rarely produced. At present it seems advisable to follow Jørstad (3) and call the whole complex *Melampsora epitea* (Kunze & Schmidt) Thuem., regardless of aecial host. As our knowledge increases it will probably be possible to set up several varieties on the basis of host relationship and small morphological distinctions. The only alternative seems to be to make species of the forms on almost every willow.

FIG. 3 and the following discussion apply particularly to the rust on *Salix arctica* and *Saxifraga rivularis*, in which all the illustrated complexities of the life cycle were demonstrated at Chesterfield Inlet. It is, however, fully applicable to any other arctic forms that possess an aecial stage, since it is fairly certain that *Saxifraga* spp. are the only aecial hosts beyond tree line; but for many forms no aecial connection has been demonstrated, and in some the ability to produce one may have been lost.

The telia form in late summer and germinate in the spring. The basidiospores infect the *Saxifraga* plants, probably in the apical meristem just as growth starts, and produce systemic-perennial mycelia. Because pycnia are produced in abundance before the aecia appear it seems reasonable to assume that the mycelium is monocaryotic at least in the aerial parts, but no cytological study has been made. The aeciospores infect *Salix* leaves, producing a dikaryotic mycelium from which may develop uredinia or telia. The

uredinia produced from aecia probably do not often sporulate early enough to produce many secondary uredinia but some of the spores sift or grow under the scales of the newly formed winter buds, forming an overwintering mycelium in the embryonic leaves or catkins The mycelium grows with the bud tissues in the spring and produces large or multiple uredinia early enough in the season to permit the production of secondary uredinia and of telia in some quantity. This device is very similar to that by which the dikaryon over-winters in *Chrysomyxa,* but only uredinia are produced in the spring. Some aeciospores may also possibly give rise to overwintering mycelia, but probably the majority are shed before the willow terminal buds are formed. Here we have three methods of overwintering, and three interlocking cycles, in a single rust. First, the monokaryon can persist year after year in the absence of *Salix,* although with no spread. Second, the dikaryon can persist in the winter buds, producing uredinia and permitting spread but no genetic change. Finally, the full cycle, with gene recombination and spread, is possible when the two hosts are contiguous.

The ability of *M. epitea* to overwinter in the willow buds was mentioned by Jørstad and was obvious in some of the specimens received in 1948. The fact that the mycelium is systemic-perennial in *Saxifraga* is also of prime importance, but it seems to have received no recognition in the literature. Observations at Great Whale River convinced me that tree line was about the limit for the conventional long-cycled rust. It was disconcerting to receive, soon afterward, heavily rusted *Saxifraga oppositifolia* from Cornwallis I., roughly a thousand miles beyond tree line. The habit of *S. oppositifolia* is such that the systemic condition was not obvious in these specimens, although it is clear to me now. The puzzle was not resolved until infected plants were seen in the field at Chesterfield Inlet.

Although *M. epitea* was moderately common on *Saxifraga rivularis* and occurred sparingly on *S. cernua* and *S. oppositifolia,* at least 90 per cent of the rust in *Salix* colonies at Chesterfield Inlet originated from the winter buds. At Great Whale River and St. Anthony all rust appeared to result from bud infection. The available specimens suggest that the aecial stage of the forms on arctic

willows occurs most frequently north of tree line, probably because that is where the susceptible saxifrages are most abundant. Specimens of *M. epitea* in its various forms have come in abundance from almost all northern stations from which phanerogamic collections have been received. It has been seen on *Saxifraga* from 75° N., and on *Salix* from about 82° 30′ N. in northern Ellesmere I., almost the northernmost land in the world. In the vicinity of Chesterfield Inlet it occurred on seven out of thirteen *Salix* spp. and was abundant on several. Clearly it is a most successful species, and the modifications in its life cycle have played no small part in its success.

ACKNOWLEDGMENTS

The personal observations drawn upon for this paper were made during field work with the Northern Insect Survey sponsored by the Defense Research Board of Canada. The majority of the pertinent collections made by others were also taken by members of Northern Insect Survey parties. Without this sponsorship any real understanding of the rust flora of the Canadian arctic would still be impossible.

SUMMARY

The northern limit for heteroecious, self-sterile, long-cycled species of *Puccinia* and *Uromyces* is approximately tree line. Self-fertility and short-cycling enable many species to persist in the arctic on hosts of suitable habit. In *Chrysomyxa,* overwintering of dikaryotic mycelium in evergreen leaves permits simultaneous development of uredinia and telia, and also makes the rust independent of the aecial host. *Hyalopsora aspidiotus* overwinters both by amphispores and by mycelia in the aecial host, thereby producing aecia, uredinia and telia nearly simultaneously. *Melampsora epitea* overwinters as a systemic-perennial monokaryon in *Saxifraga* and as a dikaryon in *Salix* buds, as well as by telia, thus being able to survive even when the sexual part of the life cycle cannot be completed.

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LARGE-BROWN-SPORED HOUSE-ROT FUNGI IN THE UNITED STATES

Ross W. Davidson and Frances F. Lombard [1, 2]

(with 4 figures)

Merulius lacrymans Jacq. ex Fr. has never been considered a common or extremely destructive fungus in North America. It is recorded by Burt (4) as occurring here and has been encountered occasionally in houses. *Poria incrassata* (Berk. & Curt.) Burt, a closely related fungus and originally placed in the genus *Merulius,* is more common as a cause of house decay (1, 9, 10, 13). In 1946 Buckland (3) listed and illustrated cultures of *Merulius* sp. isolated from brown rot in Western red cedar trees, and Bier *et al.* (2) reported *Merulius* sp. from similar rot in fir in 1948 in western Canada. *Merulius* sp., similar to the one described by Buckland, has been obtained frequently from spruce and balsam fir trees in eastern Canada and Maine. These findings have led to a more intensive study of cultures of the large-brown-spored species of *Merulius.*

DIFFICULTY OF IDENTIFICATION

Sporophores of the fungi considered here are seldom present in association with decay in trees and are almost never included with wood specimens sent in from decay in buildings. The first fresh sporophore of *Poria incrassata* encountered by the senior writer was on the under side of a large Douglas-fir log in the woods. It was not recognized as this species because in the fresh condition it was not brown or dark in color. It was a well-developed, light gray, poroid layer and the spores were only slightly colored when

[1] Senior Pathologist and Associate Pathologist, respectively, of the Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

[2] The authors are indebted to Dr. J. D. Diller and others for decay specimens and to other laboratories for some of the cultures used.

mounted in KOH solution. Usually when fruiting is found in buildings it is old and at least partially dried up and very dark.

In contrast, *Merulius lacrymans*, when obtained in good fruiting condition, is bright red or rust brown from the copious spore deposit. Under the microscope, spores are uniformly golden brown. However, other thinner species of the large-brown-spored *Merulius* group are difficult to separate from *M. lacrymans* unless fruiting bodies are in good condition. Spores of several species are similar in size and color. Sporophores of *Coniophora puteana* (Schum. ex Fr.) Karst. (*Coniophora cerebella* Pers.) might also be confused with *Merulius* species if not obtained in excellent condition.

After studying cultures of many wood-rotting fungi, the writers have been able to readily distinguish cultures of *P. incrassata* and *C. puteana* from *Merulius* spp. Evidence is presented to show that cultures of *M. lacrymans* may also be distinguished from cultures of a common thin species, *M. americanus* Burt. It is believed that the species of *Merulius* obtained from eastern Canada and Maine may be *M. americanus*.

CULTURAL CHARACTERISTICS [3]

Key to Cultures

1. Growth fast [4] (more than 90 mm. diameter in two weeks), mycelium buff to brown ..... *Coniophora puteana*
1. Growth medium (60 to 90 mm. diameter)
   2. Mat irregularly silky or with radiating silky segments, mycelium white ..... *Poria incrassata*
   2. Mat uniformly fluffy, somewhat lobed, with or without zonations, mycelium white or white with some scattered yellow color ..... *Merulius americanus*
1. Growth slow (30 to 50 mm. diameter), mat raised fluffy, mycelium white or with scattered yellow color *Merulius lacrymans*

[3] Explanation of methods used in studying wood-rot fungi in culture has been published (7).

[4] Growth is at a room temperature of about 25° C on two per cent Fleischmann's diamalt agar.

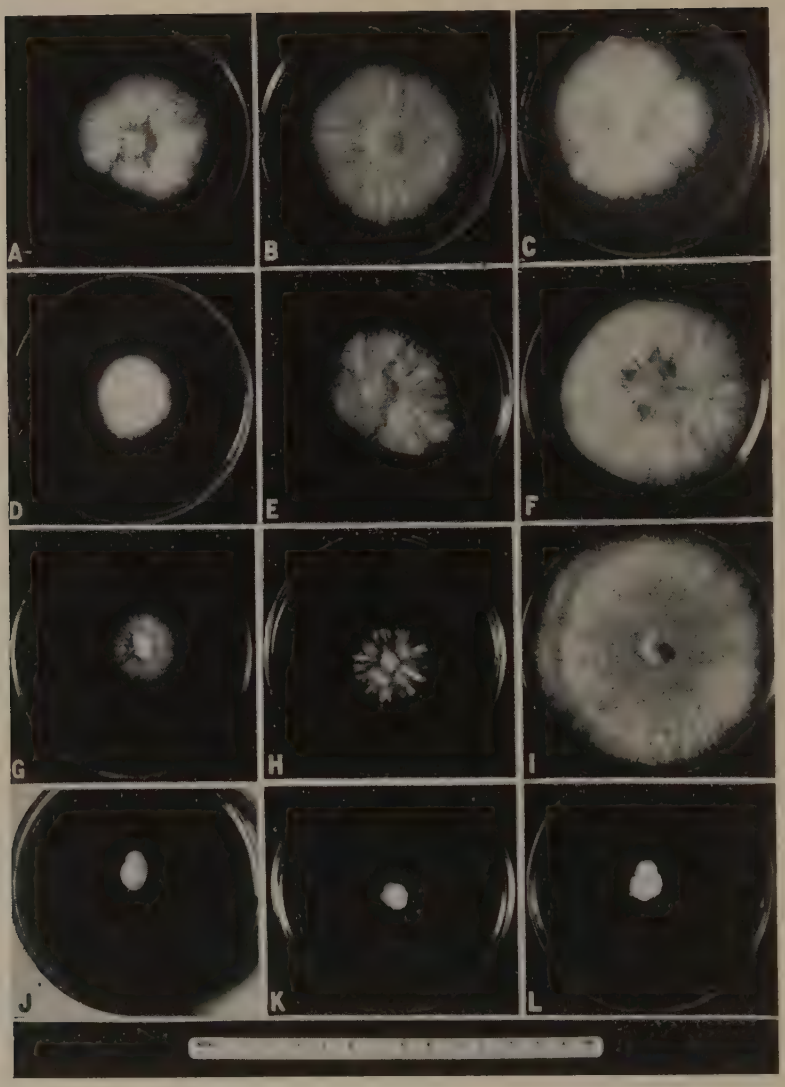

FIG. 1. One-week-old cultures grown on 2 per cent malt agar at room temperature. *A* to *C*, *Merulius americanus*, from Maryland and Georgia; *D* to *F*, *M. americanus*, from Maine and eastern Canada; *G* and *H*, *Poria incrassata*; *I*, *Coniophora puteana*; *J*, *Merulius lacrymans*, from Europe; *K* and *L*, *M. lacrymans*, from the United States.

CONIOPHORA PUTEANA (Schum. ex Fr.) Karst.

(FIG. 1, I; FIG. 2, G and H; FIG. 3, A)

Key pattern.[5] B-O-F-1-11-16.

(See published description (**5**).) Twenty-eight isolates.

MERULIUS AMERICANUS Burt

(FIG. 1, A–F; FIG. 2, A and B; FIG. 3, B; FIG. 4, A)

Key pattern. B-O-M-1-10, A-O-M-1-10, and B-O-M-1-10-16.

Growth characteristics. Growth medium, forming a mat 32–58 mm. in diameter in 7 days, 60–90 mm. in 14 days; white, occasionally with faint "citron yellow" [6] color on the inoculum or scattered around it at 7 days, usually with pale "citron yellow" or "primrose yellow" (rarely "buff-yellow" to "orange cinnamon") on or near the inoculum or localized on zonation lines in the central zone by 14 days; generally with thin fluffy growth mounded over the inoculum, mat proper intermediate to raised, uniform to margin, of radiating silky-downy hyphae at first, later fluffy-downy mycelium almost obscures the skeinlike silky-downy hyphae underneath except near the inoculum, with or without zonations, occasionally with conspicuous colorless guttulation drops on or near the inoculum, margin distinct, even or lobed, finely fimbriate; some isolates staining the agar a yellow color, which shows through the mycelium somewhat; odor strong, penetrating, disagreeable; negative oxidase reaction,[7] growth at 7 days on gallic acid medium 14–22 mm. in diameter, no growth to trace on tannic acid medium.

Hyphal characteristics. Hyphae straining, thin-walled, 2–7 (– 10)μ in diameter, with abundant clamps on large and small

[5] The key pattern has been explained previously (**7**, pp. 9–13). The first letter represents color of mat (A, white; B, white, then yellow or brown; C, yellow, etc.). The second letter of the key pattern represents positive (P) or negative (O) oxidase reaction. The third letter indicates growth rate. The numbers indicate presence of certain microscopic features such as clamps, chlamydospores, conidia, etc. The key pattern is useful in arranging the fungi, based on their characteristics in culture.

[6] Colors in quotation marks according to Ridgway (**14**).

[7] Fungi causing brown carbonizing rot in wood usually give no reaction when grown on agar containing gallic or tannic acid whereas white rot fungi cause a dark color reaction when grown on such media (**6**).

hyphae, sometimes irregularly thickened septations and walls in some empty hyphae, and occasionally more or less globular staining swellings or masses seen on some submerged hyphae; crystals octohedrals and many small angled thin plates or lumps.

Test-tube cultures. Mycelium fluffy-downy on agar slant, white, silky-downy against test tube below slant with a few to numerous small more dense strands of hyphae; yellow to "orange cinnamon" above; some cultures having numerous guttulation drops.

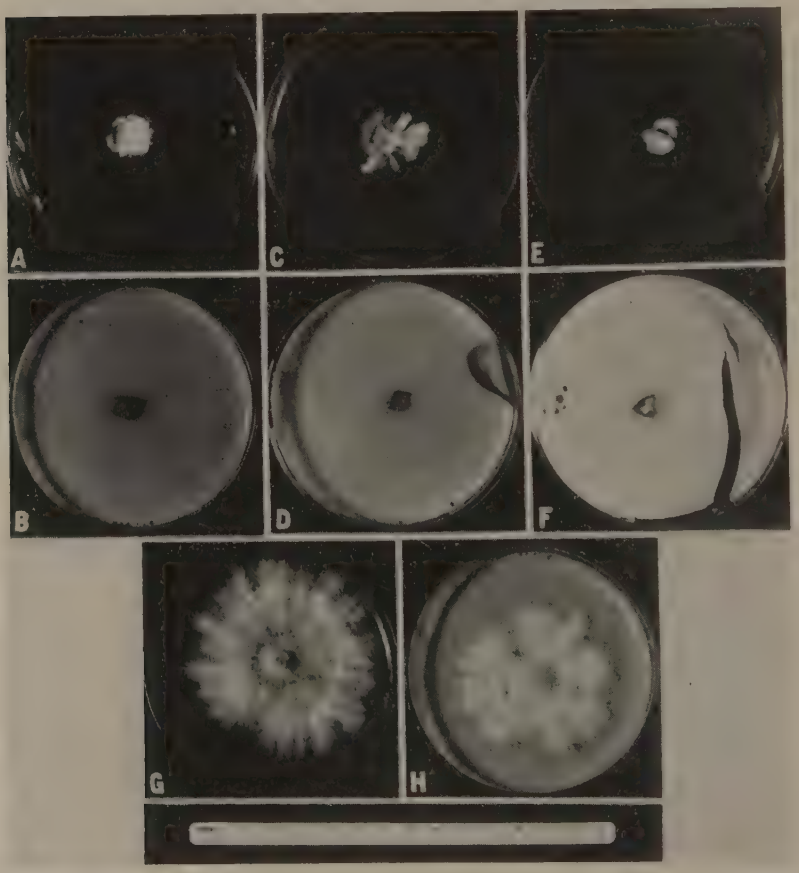

FIG. 2. One-week-old cultures on gallic and tannic acid agars grown at room temperature. *A, C, E,* and *G,* gallic acid; *B, D, F,* and *H,* tannic acid. *A* and *B, Merulius americanus; C* and *D, Poria incrassata; E* and *F, Merulius lacrymans; G* and *H, Coniophora puteana.*

FIG. 3. Twenty-eight-day-old test-tube cultures on malt agar. *A, Coniophora puteana; B, Merulius americanus; C, Poria incrassata; D, Merulius lacrymans.*

Type of decay. Brown rot of coniferous wood. Twenty-five isolates.

Remarks. The above description specificially applies to cultures obtained from a species that fruits commonly on pine logs in the woods in the vicinity of Beltsville, Md. These sporophores seem to fit Burt's description of *Merulius americanus,* but it is believed that *M. himantioides* Fries and *M. brassicaefolius* Schw. may not be specifically distinct. Also it appears that *M. silvester* as described by Falck is the same as *M. americanus.* For the time being these will have to be regarded as synonyms of one species or as a species complex that will require much more detailed study before they can be distinguished culturally. At present it is not clear which name should be used if they are to be regarded as a single species.

The various isolates from spruce and balsam fir in eastern Canada and Maine show considerable variation in growth rate but appear to be applicable to the same species as the one collected on pine logs at Beltsville. The sporophores developed on pine logs in late fall (October and November). If the eastern Canada fungus is the same, sporophores should also be common there on old infected logs in late fall. Sporophores have been collected several times under houses during the winter season.

PORIA INCRASSATA (Berk. & Curt.) Burt

(FIG. 1, G and H; FIG. 2, C and D; FIG. 3, C; FIG. 4, B)

Key pattern. A-O-M-1-10, A-O-M-1-4-10, B-O-M-1-10, and A-O-M-1-10-16.

Growth characteristics. Growth moderately rapid, forming a mat 23–38 mm. in diameter in 7 days, 62–71 mm. in 14 days; white, occasionally with small patches of "naples yellow" to "straw yellow" on the inoculum or sparingly around it at 14 days; appressed to intermediate, uniformly fine thin short downy to the margin of growth or, more generally, with small skeins arising near the inoculum or in the central zone or at the margin of growth and extending as radiating, fast-growing, silky mycelium, in some isolates ending abruptly in short raised isolated tufts and in other isolates spreading in silky fanlike sectors within mats of otherwise uni-

formly fine downy growth at 7 days, becoming more or less appressed in the central zone and raised at the margins, with the skeins and fanlike sectors (where present) still distinct at 14 days, zonate or azonate, fragile, adherent, sparse cottony aerial mycelium over the inoculum, margin either thin, even, and almost indistinct or fimbriate and distinct where silky mycelium present; odor medium to strong pungent; negative oxidase reaction, growth at 7 days on gallic acid medium 17–28 mm. in diameter, no growth to trace on tannic acid medium.

Hyphal characteristics. Hyphae staining, thin-walled, 1.7–4.7 (−8) μ in diameter, averaging about 2.5–3.5 μ, with abundant simple clamps on both large and small hyphae, rarely double clamps, occasionally in some isolates submerged hyphae have elongated thin-walled swellings that stain readily; oidia, few to many in the areas of slow-growing downy mycelium, of varying lengths; crystals large and medium octohedrals and thin plates.

Test-tube cultures. In 4 weeks mycelium yellow to buff or light brown and appressed on agar slant around inoculum (with agar showing through), white dense silky strands or dense mat developing lower down between glass wall of tube and agar; sometimes small dense powdery cushions of mycelium developing around the inoculum or at upper end of agar slant.

Type of decay. Causes brown rot of both hardwood and coniferous wood in houses and other wooden structures, and very occasionally infects logs in the woods. Fifteen isolates.

Remarks. It is present occasionally although not abundant in houses but is very destructive when present under favorable moisture conditions. This fungus is probably the only one of the group included in this paper that has not been reported in Europe. It is more prevalent in the eastern United States but occurs in the West also.

MERULIUS LACRYMANS Jacq. ex Fries

(FIG. 1, J–L; FIG. 2, E and F; FIG. 3, D; FIG. 4, C)

Key pattern. B-O-S-1-10 and B-O-S-1-10-16.

Growth characteristics. Growth slow, forming a mat 11–19 mm. in diameter in 7 days, 27–40 mm. in 14 days; white, usually with

patches of "citron yellow" color over or around the inoculum block; growth on inoculum raised, of densely mounded fine fragile downy mycelium extending upward toward the lid of the dish and laterally to almost hide the surface mat at first, merging gradually into a

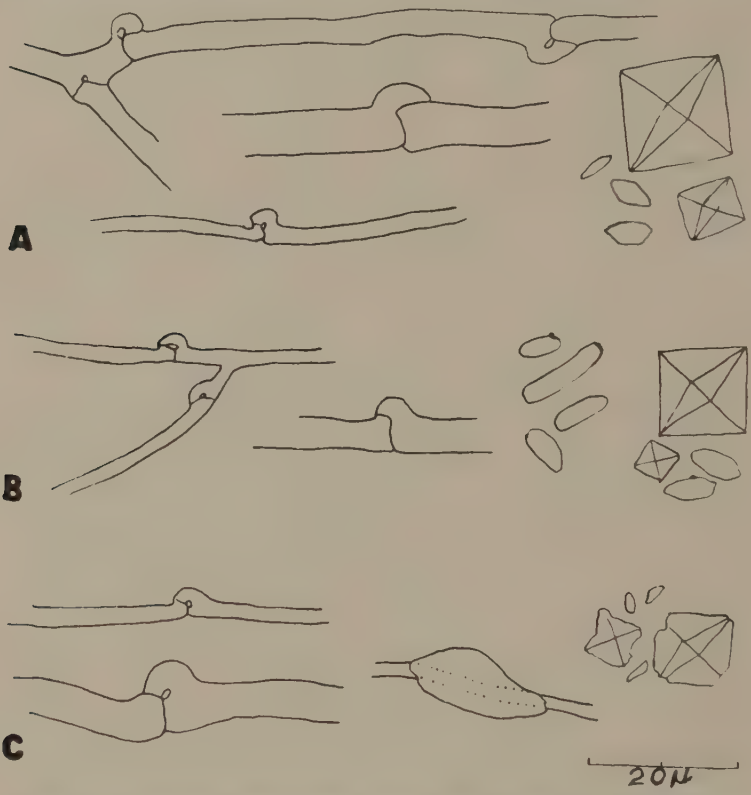

FIG. 4. *A, Merulius americanus:* hyphae and crystals. *B, Poria incrassata:* hyphae, oidia, and crystals. *C, Merulius lacrymans:* hyphae, staining mass, and crystals.

fluffy more downy-cottony less raised growth toward the margin, fragile but slightly firm underneath, azonate, margin distinct, fimbriate, even or lobed; odor faint or none; negative oxidase reaction, growth at 7 days on gallic acid medium trace to 13 mm. in diameter, 0 to 13 mm. on tannic acid medium.

Hyphal characteristics. Submerged and superficial hyphae staining, thin-walled, 2–7(−9.5)μ in diameter, with numerous

clamps on large and small hyphae; occasionally submerged hyphae with slight swellings or masses that stain; crystals numerous, octohedrals and small angled plates. According to Nobles (12), haploid cultures have no clamp connections and numerous oidia.

Test-tube cultures. In four weeks growth at ordinary room temperature is rather limited, covering agar slant and growing only slightly onto upper part of agar cylinder, uniform fine fluffy-downy mat, white or with some "citron yellow" color.

Type of decay. Brown rot of coniferous wood. Three isolates.

Remarks. This fungus is not as common as *Poria incrassata* in this country but does occur occasionally in buildings in which moisture conditions are favorable and the temperature relatively low.

TEMPERATURE RELATIONS

As shown in TABLE I, three of the four fungi studied have intermediate and the other one (*Merulius lacrymans*) low temperature requirements for optimum growth. The species have not shown

TABLE I

FUNGUS GROWTH ON MALT AGAR, MEASURED AT THE END OF ONE WEEK AT CONSTANT TEMPERATURES

Name of fungus	Average diameters of growth at constant temperatures									
	18° C	20° C	22° C	24° C	26° C	28° C	30° C	32° C	34° C	36° C
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
Coniophora puteana	76*	82	87	90+	90+	90+	66	14	trace	0
Merulius americanus	34	30.6	41	45.6	47.5	47.5	33.5	23	trace	0
Poria incrassata	20	24	26	43	42	40	35	20	trace–0	0
Merulius lacrymans	27	28.5	21	23	16	trace	0	0	0	0

* Mat diameters are averages of several growth tests.

a very wide range of temperature requirements among the different isolates. This was the case with *M. lacrymans,* although only one European and two American isolates were available for study. All of the cultures show a rather wide optimal temperature range.

The principal point of difference between these results and those of Humphrey and Siggers (11) is that their one European isolate of *M. lacrymans* had an intermediate temperature requirement.

It is possible that such variation could result from differences between strains of a species. If further careful studies show such wide variation to be common, separation by temperature requirements alone would be questionable. So far in our experience this has not been shown to be true and any proof of wide variation within a species must be verified by accurately identified sporophore cultures.

DISCUSSION

The four species can be differentiated readily at four days of age on malt dishes. *Coniophora puteana* makes the fastest growth and the mat is cream-colored, intermediate to raised, and mainly downy to downy-cottony composed of fine or coarse strands of hyphae radiating from the inoculum block. *Merulius americanus* is next in growth rate and the mat is white, with or without a tinge of lemon yellow near the inoculum, and is mostly appressed, composed of bunches of silky-downy radiating and ascending hyphae with thin but highly raised growth over the inoculum. *Poria incrassata* is slightly slower growing than the above species, making scant growth over the inoculum block; the mat is appressed and composed of very short, slightly ascending, downy mycelium with the characteristic dense radiating silky growth just beginning at the margin of the mat. *M. lacrymans* is the slowest in growth rate and the mat is white, consisting mainly of raised fluffy dense growth over the inoculum, with scant growth on the surface of the agar.

Two of the four fungi described in this paper, *P. incrassata* and *C. puteana,* are quite destructive in houses and other wooden structures in the United States. The other two, *M. lacrymans* and *M. americanus,* are present only occasionally in buildings, and the latter is not known to cause extensive destruction. *M. americanus* and *C. puteana* are common on trees or logs in forested areas, whereas the other two are not known to be common in the forests.

All but *P. incrassata* may be common in Europe. An examination of Falck's 1912 paper (8) suggests that his three large-brown-spored species may be the same as the three studied here. Our *M. lacrymans* should be his *M. domesticus* and *M. americanus* may be the same as *M. silvester.* Most certainly the *C. puteana* in

houses in this country is the same as the fungus Falck described under the name *C. cerebella.*

It is probable that there are additional large-brown-spored *Merulius* species in this country besides the two mentioned in this paper but up to the present time the writers have not encountered any that is clearly culturally distinct.

SUMMARY

Cultures of *Merulius lacrymans* have been distinguished from cultures of other wood-rotting fungi and from cultures of the common species *Merulius americanus.* The two species of *Merulius* may be separated by differences in growth rate and mycelial mat characteristics when grown on 2 per cent malt agar in 90 mm. Petri dishes. *M. lacrymans* grows best at a temperature of about 18° C and does not grow at 28° C. *M. americanus* grows best at 26° C, grows well at a temperature of 30° C, and will even make a slight growth at 34° C. These *Merulius* species have been encountered a few times in houses.

Coniophora puteana and *Poria incrassata* are two species causing destructive decay in houses in this country; they also have large brown spores. However, *C. puteana* is a much faster growing fungus than the *Merulius* species and *P. incrassata* may be differentiated from the other species by its mat and type of growth. Temperature requirements for *C. puteana, P. incrassata,* and *M. americanus* are quite similar.

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SPECIES OF SYNCHYTRIUM IN LOUISIANA. VIII. NEW SPECIES

Melville T. Cook

(with 51 figures)

The species of the genus *Synchytrium* cause the formation of galls or abnormal growths on the leaves, petioles, stems and sometimes on flowers of the host plants. Most species of *Synchytrium* attack but one species of host plant. The external characteristics of some of them and the fact that they are specific enable the student to identify many species with some degree of certainty and without difficulty. The descriptions of 43 of 95 species reported from the northern hemisphere are incomplete but they very definitely belong to this genus. Some of the characters used in the descriptions, such as size and color of fungus, are of very little value; the size is variable even in the mature stages and the colors are variable with age and are practically the same in all species. The zoospore is hyaline; the young fungus becomes light yellow soon after infection and then gradually orange, red and sometimes black with age.

In some species the sori can be seen as yellow or orange spots without difficulty, while in some others they can be seen only by the aid of a hand lens.

The fungus in some species stains so deeply that the structure cannot be seen without destaining. In these cases the stain usually disappears from the host tissues far in advance of the stain in the fungus. In some species the fungus does not overstain readily. The shape and structure of the galls is more definite and valuable for descriptions and identification than any characters of the fungus. In this paper, more attention is given to the galls than to the fungi. The descriptions of the 43 species which are incomplete do not contain any information concerning the sporangia. These data are very difficult to obtain in some cases, because the fungus becomes separated from the host tissues and falls out of

the cavity before the formation of the sporangia. The galls are very conspicuous in some species while in others they are very inconspicuous, causing, in fact, scarcely any deformity of the host tissues.

Although the galls are very definite in shape, they may show wide variations. A gall in contact with a vein may be quite different from one that does not have such a contact and the writer has usually classed them as abnormal or deformed galls.

Infection of the trichomes is very rare. The zoospore has been observed to enter the stomata in a few cases but without the formation of a gall. The period of infection is previous to the formation of the palisade. There are a few records in the literature of sub-epidermal infections but the author is inclined to doubt these studies. The infections in all species studied by the writer are in the epidermal cells, usually in the lower epidermis. The writer has never observed infections in sub-epidermal cells. In one species (*S. oxalidis* Cook) overgrowths gave the appearance of sub-epidermal infections, but this was due to overgrowths. The infected host cell reacts very quickly after the penetration by the zoospore. In a few cases the fungus increases in size for a time and then become dormant in the tissues of the host plant (FIGS. 17, 39). This is probably due to a late infection of the host cell which does not react. The cells of the galls are frequently infected when very young; the result is the formation of compound galls.

Infections occur during periods of wet weather, which may be very short. In Louisiana the infections occur in the late winter or spring following rains which may flood areas for a very few hours. The infections occur on very low herbaceous or very young and very small woody plants.

The descriptions of species previously reported by the author have contained descriptions of the sporangia. The sporangia have not been studied in the species reported in this paper because it is necessary for the writer to discontinue this work. However, the data are sufficient for the identification of the species.

The author has described several species of *Synchytrium* collected in Louisiana. In many cases distinct species will be found on different host plants but growing very close together. This

has raised the question as to the possibility of some of these species attacking several different species of host plants. If that is true, some of my species should be reduced to synonyms. Of course, this question cannot be answered definitely without making cross-inoculation experiments, which the writer is not in a position to do at this time. However, we have certain facts to support the writer's contention that most or possibly all of them are distinct species. The literature indicates that of 95 species previously reported, 40 species occur on one species of host plants only, 6 species occur on two species of host plants, 4 species on three species of host plants, 2 species on four species of host plants and one species on six species of host plants. This indicates that most species of *Synchytrium* are specific in their attacks on host plants. The one exception to this conclusion is *S. aureum* which has been reported on 197 species of host plants, but these records are questionable. *S. aureum* is most likely a composite of many species. Furthermore, the writer has shown that in some cases two species of host plant grow in contact and only one species is attacked by the fungus. An example of this is *S. stellaria* which attacks *Stellaria media,* and *S. cerastii* which attacks *Cerastium viscosum.* These two species of host plants are frequently found growing in contact and only one infected. The same has been noted on other species but the observations have not been so numerous. In some species the shape and structure of the galls are so definite as to indicate the species. This is in harmony with the writer's studies on galls caused by insects, many years ago, in which it was found that the structure of the galls depended on the species of insect and not on the host plant.

Synchytrium Parthenocissi sp. nov.

Gallis post contagionem celerrime ortis; in utraque superficie foliorum sitis; per spatium circiter aequale ultra alteram superficiem se extendentibus; colore subviridi ad colorem purpureum; saepimentis leviter demutatis aut absentibus; cavis subsphaericis; foramine claro; 304–760 μ. Fungus cellulam primo sed haud serius implet; 15–248 μ diametro. Hab.: *Parthenocissum quinquefolia* (L.) Planch.

Galls on both surfaces of leaves, nearly spherical; may extend about equal distance on either surface but usually most prominent on lower surface; the palisade is slightly modified; when the pene-

tration is on the upper surface, the palisade is broken, shortened or absent, structure rather dense; cavity nearly spherical; cells around cavity are modified very quickly; greenish to purple in color; sometimes concave above with papillae in center; opening distinct; the gall grows very rapidly and in advance of the formation of the fungus but the leaf begins to thicken very soon after infection. The fungus fills the cell completely at first (FIG. 1) but after a time the cell cavity grows more rapidly than the fungus (FIG. 2). Wall around fungus is very thin. The cell material surrounding the fungus is thick and stains very dark (FIGS. 1, 2).

Similar infections which may be caused by the same species are found on *Ampelopsis arborea* (L.) Desmoulins and *Vitis* sp.[1]

Synchytrium Duchesneae sp. nov.

Gallis in utraque superficie foliorum; insitis aut in superficie foliorum manentibus; 450 × 600 vel 600 × 750 μ. Fungus 95 × 300 μ. Hab.: *Duchesnea indica* (Andr.) Focke. Not abundant. Baton Rouge, Louisiana, U.S.A.

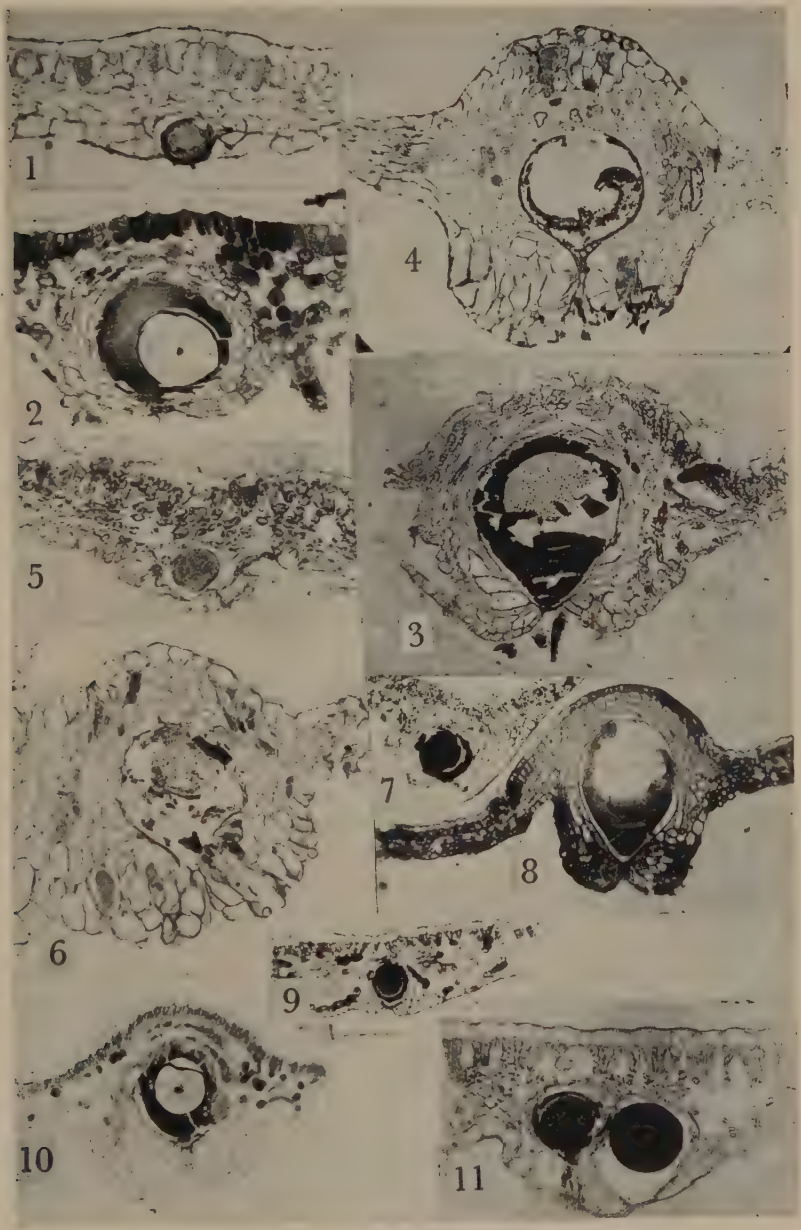
Leaf structure very loose. Galls on leaves and petioles; large, projecting on both surfaces of leaves with opening mostly on under surfaces; may rest on surface of leaf or be submerged in tissues of the host; green; galls grow more rapidly than the fungus; cavity filled by fungus at first but not later; very little modification of cell structure of leaf but similar to *S. Parthenocissi;* surface of young cavity usually spherical; opening very distinct.. Wall around fungus thin. Palisade usually absent in mature gall. FIGS 5, 6.

Synchytrium Corni sp. nov.

Gallis in utraque superficie foliorum sitis; in parte inferiore maxime exstantibus; sparsis; hemisphaericis; viridibus; 180 × 375 vel 525 × 525 μ.

[1] Galls caused by *Phylloxera vastatrix* are common on *Vitis* spp. but are easily recognized.

FIGS. 1–4. *Synchytrium Parthenocissi.* 1. Early infection on lower surface of leaf. Early stage of gall formation. 2. The sorus ready for division; gall of small cells; content of infected cell prominent. 3. Gall composed of small cells. 4. Mature gall; opening distinct. FIGS. 5–6. *Synchytrium Duchesneae.* 5. Early infection. 6. Mature gall. FIGS. 7–8. *Synchytrium Corni.* 7. Early infection. 8. Mature gall. FIGS. 9–10. *Synchytrium Asterum.* 9. Early infection. 10. Advanced stage; sorus ready to divide; content of infected cell prominent; gall composed of small cells; palisade intact. FIG. 11. *Synchytrium Epihaleri.* Two infected cells; one on left with well pronounced tube; palisade not injured.



FIGS. 1–11.

Fungus clarissimus, 150 × 250 μ diametro. Hab.: *Cornus Drumondi* Meyer, Baton Rouge, Louisiana, U.S.A.

Leaf structure dense; galls on both surfaces of leaves; sometimes abundant but usually scattered; greenish; hemispherical and most prominent below; concave above with elevation in pit; opening distinct. Palisade undeveloped. Fungus usually showing through on under side. Wall around fungus very thin. FIGS. 7, 8.

Synchytrium Asterum sp. nov.

Gallis parvis; in utraque superficie foliorum; plerumque sphaericis; colore viridi usque ad colorem purpureum; 225 × 330 μ vel 300 × 300 μ. Fungus 105 μ diametro. Hab.: *Aster* sp., Baton Rouge, Louisiana, U.S.A.

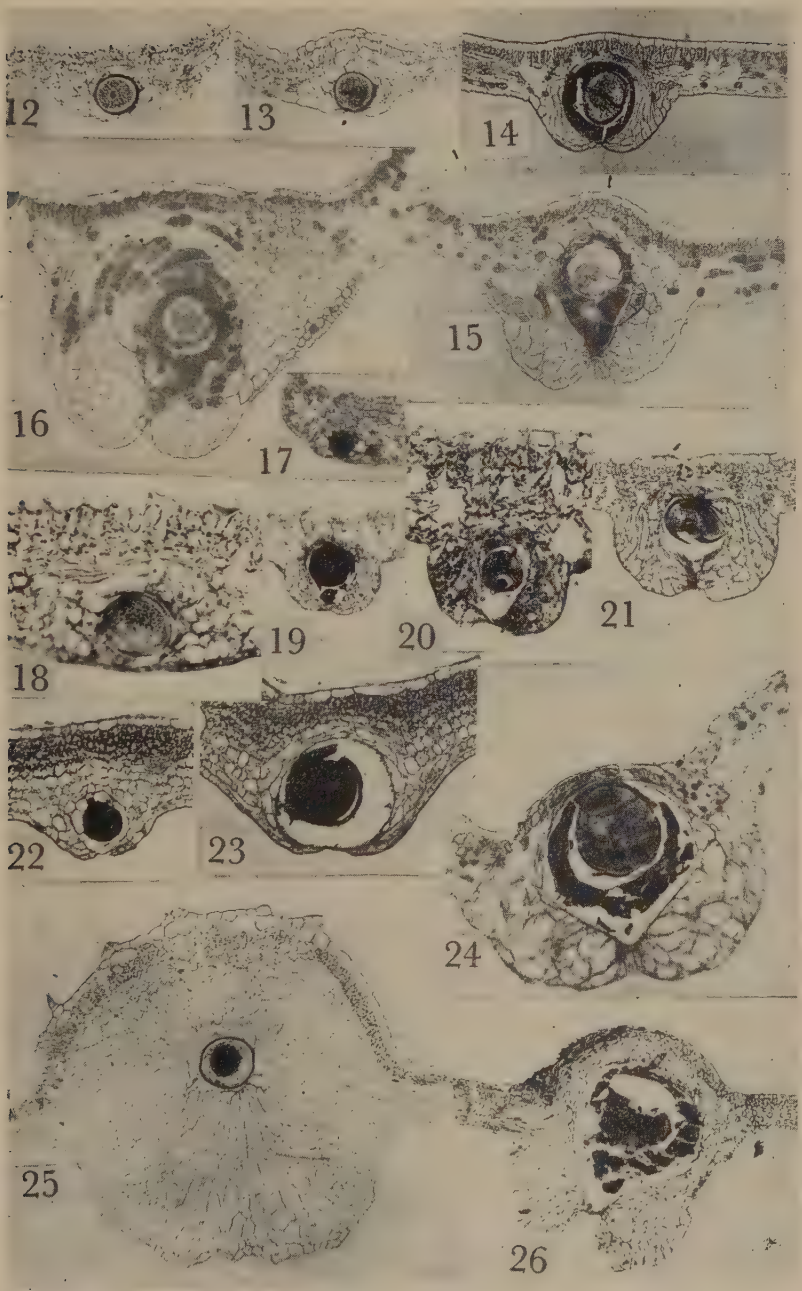
Leaf structure very loose; galls small; on both surfaces of leaves; usually hemispherical; green to purple; opening usually on lower surface; content of host cell very dense and deep staining; wall around fungus thick; plants too young to determine species. FIGS. 9, 10.

Synchytrium Cocculi sp. nov.

Gallis plerumque in una superficie foliorum sitis; basi in textibus hospitis insita; viridibus; 150 × 525 μ. Fungus in folio partim insitus; flavus aut ruber; 60 × 150 μ. Hab.: *Cocculus carolinus* (L.) DC., Baton Rouge, Louisiana.

Leaf structure very loose; galls usually small and on one surface of the leaf or the basal part embedded in leaf; convex on both sides but most prominent on lower surface; spherical on stems; may rest on surface or be partially embedded in tissue; green; cavity connected with the outside by long or short tube. Fungus yellowish to brownish red; wall around sorus very thick. FIG. 11.

FIGS. 12–17. *Synchytrium Cardiospermi.* 12. Very early infection. 13. Slightly advanced. 14. Further advanced, showing substance in infected cell. 15. Well advanced; infected cell cavity pear shaped; content of infected cell prominent. 16. Further advanced. 17. Late infection in which growth of sorus has stopped and gall not formed. FIGS. 18–21. *Synchytrium Ilicicola.* 18. Early infection showing thickening of leaf and formation of wall around sorus. 19. Early stage in gall formation. 20, 21. Advanced stages in gall formation. FIGS 22–24. *Synchytrium Liquidambaris.* Stages in formation of gall. FIGS. 25–26. *Synchytrium Gonolobi.* 25. Advanced stage showing that the gall is very large and the sorus very small. 26. Mature gall.



FIGS. 12–26.

Synchytrium Cardiospermi sp. nov.

Gallis in utraque superficie foliorum sitis, numerosissimis autem in inferiore; 301 × 75 vel 375 × 525 μ. Fungus 27–120 μ diametro. Hab.: *Cardiospermum Halicacabum* L., Baton Rouge, Louisiana.

Leaf structure very loose; galls on either surface of leaf but most abundant on lower; gall starts very soon after infection; cavity pear-shaped in mature galls; opening distinct; fills cavity at first but not later in its development; content of cavity thick; palisade may persist or be only partially developed; green. Wall around fungus very thick (fungus may be quite large and gall quite small). Fungus may become dormant without forming gall (FIG. 17). FIGS. 12–17.

Synchytrium Ilicicola sp. nov.

Gallis numerosis; plerumque in superficiebus inferioribus sitis; in textibus hospitis partim aut omnino insitis; duris, praesertim in stirpe; 150 × 375 μ. Fungus 75–105 μ diametro. Hab.: *Ilex vomitoria* Ait. and *I. decidua.*

Leaf structure loose; galls on leaves mostly on lower surface; hemispherical below and slightly convex above; numerous; sometimes partially or completely embedded in host tissue but usually resting on surface of leaf; green when young; green to dark purple with age; becoming hard with age, especially on stems; surrounding cells may become modified quickly. Wall around fungus thick. Leaf structure slightly distorted. Fungus grows rapidly and fills cavity but grows slower than the cavity after the formation of the gall. Opening to cavity distinct. Fungus may be embedded in leaf without forming gall. FIGS. 18–21.

Synchytrium Liquidambaris sp. nov.

Gallis in utraque sed plerumque inferiore superficie sitis; sphaericis; viridibus; 150 × 150 vel 450 × 450 μ. Fungus 75–300 μ diametro. Hab.: *Liquidambar styraciflua.*

Leaf structure very dense; galls on both surfaces, mostly on lower; usually spherical below and concave above; green; opening very distinct; gall usually larger on the lower surface than on the upper surface; palisade may be destroyed; fungus fills cavity at first but not later; wall around fungus very thick; cell content prominent; fungus may lie in tissue without forming gall. FIGS. 22–24.

Synchytrium Gonolobi sp. nov.

Gallis in utraque superficie sitis; numerosissimis autem in inferiore; hemisphaericis; 600 × 600 μ. Fungus 75 μ diametro. Hab.: *Gonolobus* sp.

Leaf structure very loose; galls on either surface but most abundant on lower; hemispherical; starts immediately after infection and before the palisade is formed; opening indistinct; palisade slightly modified; 600 × 600 μ; fungus fills cavity for a time but cavity enlarges more rapidly than fungus. Content of infected cell prominent with age. Fungus small as compared with gall; wall around fungus thick. FIG. 25.

Synchytrium Carpini sp. nov.

Gallis raris; in utraque superficie foliorum, numerosissimis autem in inferiore sitis; sphaericis; 210 μ diametro. Fungus 135 μ diametro. Hab.: *Carpinus* sp.

Leaf structure loose; galls rare; more or less spherical and prominent on lower surface; opening distinct. FIG. 26.

Synchytrium Clematidis sp. nov.

Gallis in utraque superficie foliorum sitis; sphaericis; 300 × 400 vel 600 × 750 μ Fungus 90 × 150 μ diametro. Hab.: *Clematis* sp., Baton Rouge, Louisiana, U.S.A.

Leaf structure very loose; galls usually projecting on one surface or concave above and sometimes below; spherical; opening distinct; sometimes new galls are formed on epidermal cells of older galls. Both fungus and gall grow rapidly. Wall around fungus thick. Content of infected wall very thin. FIG. 27.

Synchytrium Bignoniae sp. nov.

Gallis numerosis in utraque superficie foliorum sitis; sphaericis in petiolis stirpibusque; utraque superficie aequaliter prominentibus; duris, rubris, purpureis, nigris; 300 × 300 vel 750 × 1500 μ. Fungus 75–182 μ diametro. Hab.: *Bignonia capreolata* L., Baton Rouge, Louisiana, U.S.A.

Structure of leaf very dense; galls abundant on both surfaces of leaves and on petioles and stems; mostly on lower surface of leaves; projecting about equally on both surfaces or larger on side of infection; usually spherical; large and hard; greenish, yellowish, reddish, purple, becoming black with age; cell structure compact in mature galls; cavity almost spherical; tube leading to cavity very small but distinct. Fungus fills cavity at first but not later. Wall around fungus thin. Very abundant. FIGS. 28–30.

Synchytrium Erechtitis sp. nov.

Gallis parvis; numerosis; in utraque superficie foliorum; infra hemisphaericis; in petiolis stirpibusque; 105 × 150 vel 600 × 600 μ. Fungus 90–

150 μ diametro. Hab.: *Erectites hieracifolia* (L.) Raf., Baton Rouge, Louisiana, U.S.A.

Leaf structure very loose when young; galls small; abundant on both surfaces; hemispherical below; on petioles and stems; may be embedded in leaf tissue; opening very large. Fungus fills cavity at first, but cavity grows more rapidly than fungus; opening distinct; cell structure compact and with very little modification; 90–150 μ; very pale, becoming yellowish. Wall around fungus very thick. Palisade usually absent. FIGS. 31, 32.

Synchytrium Polygoni sp. nov.

Gallis in utraque superficie foliorum; plerumque numerosis et in racemis; parvis; viridibus; 300 × 300 vel 525 × 600 μ. Fungus 90–195 μ diametro. Hab.: *Polygonum* sp., Baton Rouge, Louisiana, U.S.A.

Leaf structure very dense; galls on both surfaces of leaves; usually abundant and in clusters; small and green; palisade not present in mature galls; host cell material distinct; cavity pear-shaped; opening distinct. Fungus yellow, becoming orange. Wall around fungus thin. FIG. 33.

Synchytrium Callicarpae sp. nov.

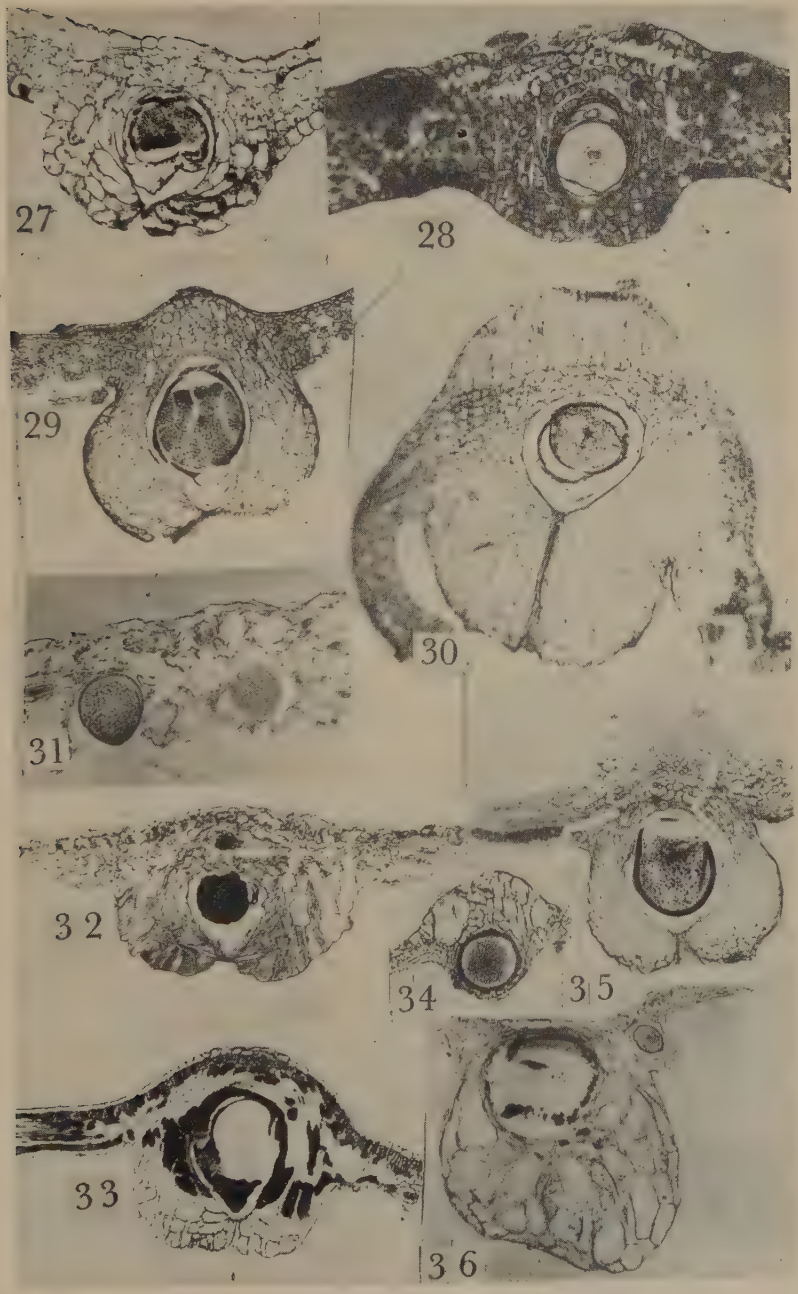
Gallis numerosis; parvis; in utraque superficie foliorum sitis; sphaericis aut elongatis; viridibus; 450 × 450 vel 525 × 525 μ. Fungus 45–180 μ diametro. Hab.: *Callicarpa americana* L., Baton Rouge, Louisiana, U.S.A.

Leaf structure loose; galls numerous, small; projecting on both surfaces of leaves; may be spherical or elongated; usually raised but frequently depressed above; infections start on lower surface but gall usually projects above; green, almost same color as leaves; leaves frequently deformed; in some cases canal to infected cell is distinct; wall around fungus very thick. FIGS. 34–36.

Synchytrium Tecomae sp. nov.

Gallis in utraque superficie sed plerumque in inferiore se extendentibus; viridibus; 300 × 450 vel 450 × 600 μ. Fungus 150 × 450 μ diametro. Hab.: *Tecoma radicans* (L.) Juss., Baton Rouge, Louisiana, U.S.A.

FIG. 27. *Synchytrium Clematidis*. Mature gall. FIGS. 28–30. *Synchytrium Bignoniae*. Stages in the formation of the gall. The sorus is much smaller than the mature gall; canal small and well defined. FIGS 31–32. *Synchytrium Erechtitis*. 31. Early stage showing the formation of the wall around the sorus. 32. Mature gall showing large opening and uninjured palisade. FIG. 33. Synchytrium Polygoni showing mature gall. The palisade is slightly injured. FIGS 34–36. *Synchytrium Callicarpae*. 34. Early stage in formation of gall. 35, 36. Later stages.



FIGS. 27–36.

Leaf structure very loose; galls on upper and lower surfaces; frequently causing a pit in the leaf; most common on lower surfaces; projecting on both surfaces; green; cavity spherical; opening distinct; palisade modified. FIGS. 37, 38.

Synchytrium Mitchellae sp. nov.

Gallis in utraque superficie foliorum sitis; maxima parte in superficiebus inferioribus sparsis; hemisphaericis; viridibus; 150 × 150 μ. Fungus circiter 75 μ diametro. Hab.: *Mitchella repens* L., Baton Rouge, Louisiana, U.S.A.

Leaf structure very loose; galls scattered on both surfaces of leaves, but mostly on lower surface, convex above and concave below; hemispherical; forms very quickly; green; may remain in tissues without causing gall (FIG. 39); fills cavity but cavity enlarges more rapidly than fungus; cell content takes stain very readily. FIGS. 39–42.

Synchytrium Trachelospermi sp. nov.

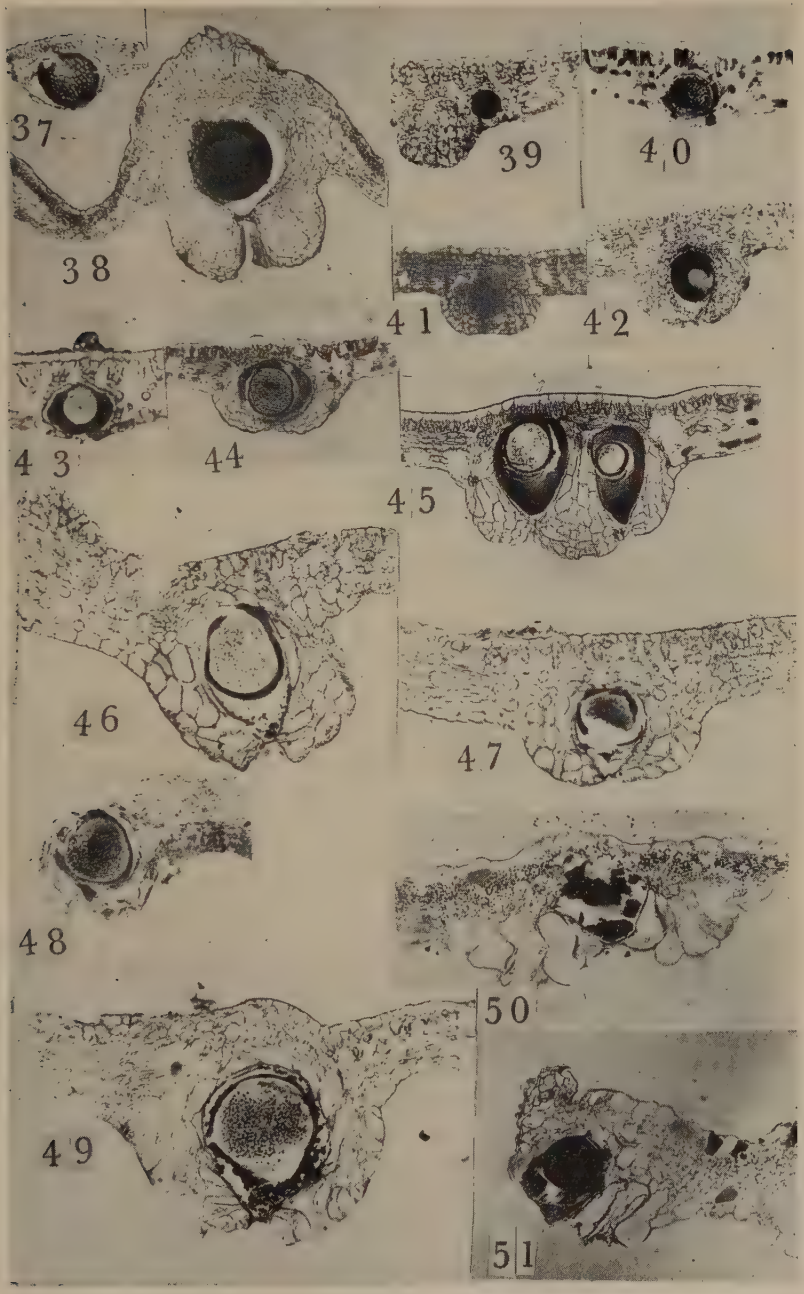
Gallis hemisphaericis; basi in textu insita; viridibus; 150 × 175 vel 600 × 650 μ. Fungus 105–180 μ diametro. Hab.: *Trachelospermum deforme* (Walt.) Gray, Baton Rouge, Louisiana, U.S.A.

Leaf structure loose; galls hemispherical below; convex above; base embedded in the tissues; green; cavity spherical becoming pear-shaped; both cavity and fungus enlarge rapidly; content of host cell dense and deep staining; wall around fungus thick. Very little change in structure of leaf. Host cell content prominent. FIGS. 43–45.

Synchytrium Fraxini sp. nov.

Gallis minimis; maxima parte in superficie inferiore foliorum sitis; colore viridi usque ad colorem nigrum; 300 × 350 vel 600 × 600 μ. Fungus 95–300 μ diametro. Hab.: *Fraxinus* sp.

FIGS. 37–38. *Synchytrium Tecomae.* 37. Early stage in formation of gall. 38. Mature gall. FIGS. 39–42. *Synchytrium Michellae.* 39. An infection without formation of gall. 40. Early stage is in formation of gall. 41, 42. Advanced stage in formation of gall. FIGS. 43–45. *Synchytrium Trachelospermi.* 43. Early stage showing that the sorus has developed very rapidly; the leaf is slightly swollen. 44, 45. Advanced stages in formation of gall. FIGS 46–47. Synchytrium Fraxini. Stages in the formation of the gall. FIGS. 48, 49. *Synchytrium Ulmi.* Early and late stages in the formation of the gall. FIGS. 50, 51. *Synchytrium Violae.* Showing gall composed of large, elongated cells.



FIGS. 37–51.

Leaf structure very loose; galls very small; mostly on lower surface; opening distinct; green to black; wall around fungus very thick. FIGS. 46, 47.

Synchytrium Ulmi sp. nov.

Gallis raris; in utraque superficie foliorum, numerosissimis autem in inferiore inventis; 225 × 255 μ. Fungus 120 μ diametro. Hab.: *Ulmus* sp., Baton Rouge, Louisiana, U.S.A.

Leaf structure loose; galls rare; projecting on both surfaces but most common on lower surface; opening prominent; wall around fungus very thick. FIGS. 48, 49.

Synchytrium Violae nov. sp.

Gallis raris; in superficie inferiore foliorum sitis; magnitudine 80 × 300 vel 80 × 375 μ variantibus; compage cellularum laxissima, basi in textu hospitis insita. Fungus circiter 105 μ diametro. Hab.: *Viola* sp., Baton Rouge, Louisiana, U.S.A.

Leaf structure rather loose; galls usually on lower surface of the leaf and variable in size, ranging from 80 × 300 to 80 × 375 μ; the basal part embedded in the host tissue; composed of very long cells; opening very distinct. Substance around fungus very distinct. Fungus about 105 μ in diameter. FIGS. 50, 51.

Synchytrium globosum Schroeter has been reported on six species of *Viola* in Europe. *S. aureum* Schroeter has been reported on three species of *Viola* in Europe and on one species in Japan. Neither of these species corresponds in measurements or structure with the species found in Louisiana.

The writer wishes to express his thanks to Dr. S. J. P. Chilton for encouragement in these studies, to Dr. C. W. Edgerton and Dr. E. C. Tims for making the photographs and to Dr. P. J. Moorehead for preparing the latin diagnoses.

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SOUTH AMERICAN SPECIES OF PUCCINIA ON SALVIA [1]

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(WITH 13 FIGURES)

This paper is a monograph of the species of *Puccinia* known to occur on *Salvia* in South America. A previous monograph by Baxter and Cummins (2) reviewed the North American members of this group of rusts. In the present paper eleven South American species of *Puccinia* on *Salvia* are redescribed and redefined. In addition, two species are described as new.

Knowledge of the South American *Salvia* rusts is based chiefly on the Holway collections and to a lesser extent on specimens collected by Mayor, Lagerheim, and others. The Sydows (8), in 1904, published descriptions and illustrations of the species known at that time. Two new species were described from Colombia by Mayor (7) in 1913, and in 1932 seven of the species treated in the present paper were described as new by Jackson and Holway (5).

The urediospores of these rusts, as in the North American members of the group, have zonate, equatorial or subequatorial germ pores, except in *Puccinia roesteliiformis* Lagerh., which is distinct in having urediospores with scattered pores. With respect to teliospore characters the South American species of *Puccinia* on *Salvia* can be divided into two groups. In one group the teliospores germinate only after a resting period and the teliospore wall is sculp-

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tured throughout, with the marking ranging from striate or finely punctate-verrucose to a coarsely verrucose or rugose type of sculpturing. The other group is characterized by teliospores which germinate without a resting period and in which the wall is thinner, paler, and, except in one species, smooth throughout.

In separating species or groups of species in the key, teliospore characters have been used almost exclusively. The most reliable of these are: (a) the time of germination, (b) the nature of the spore wall, (c) the persistence and length of the pedicel, and (d) the position of the pore in the lower cell. The only use of an urediospore character has been in separating two species on the basis of the thickness of the urediospore wall.

KEY AND DESCRIPTION OF SPECIES

Teliospores germinating without a resting period; wall smooth throughout, or smooth in the lower half and roughened above.
- Teliospore pedicel 12–23 μ wide, 160–300 μ long.
  - Teliospores 20–30 × 50–90 μ.....1. *P. astricta.*
  - Teliospores 16–23 × 100–155 μ.....2. *P. roesteliiformis.*
- Teliospore pedicel less than 12 μ wide, less than 160 μ long.
  - Teliospores 43–66 μ long.
    - Teliospores 16–23 μ wide; apical umbo smooth.....3. *P. albicera.*
    - Teliospores 20–30 μ wide; apical umbo striate.....4. *P. soledadensis.*
  - Teliospores 26–43 μ long.....5. *P. conspersa.*

Teliospores germinating after a resting period; wall striate, verrucose, or rugose throughout.
- Teliospore wall 5–8 μ thick; pedicel persistent, 100–150 μ long.
  - Pore of lower cell near the pedicel.....6. *P. gilliesii.*
  - Pore of lower cell next to the septum.
    - Urediospore wall 1–1.5 μ thick.....7. *P. aenigmatica.*
    - Urediospore wall 3 μ thick.....8. *P. porphyretica.*
- Teliospore wall less than 5 μ thick; pedicel fragile, less than 50 μ long.
  - Teliospore wall rugose, dark chestnut-brown.
    - Pore of lower cell next to the septum.....9. *P. conturbata.*
    - Pore of lower cell next to the pedicel.....10. *P. mitoensis.*
  - Teliospore wall striate or finely verrucose, light chestnut or cinnamon-brown.
    - Pore of lower cell next to the septum.....11. *P. sana.*
    - Pore of lower cell next to the pedicel.
      - Teliospores 28–37 μ long.....12. *P. sphacelicola.*
      - Teliospores 36–43 μ long.....13. *P. striatifera.*
- Teliospore wall less than 5 μ thick; pedicel persistent, 50–150 μ long.
  - Pedicel thick-walled, 100–150 μ long.....14. *P. impedita.*
  - Pedicel thin-walled, 50–80 μ long.....15. *P. farinacea.*

1. PUCCINIA ASTRICTA Jacks & Holw. Mycologia **24**: 74. 1932.

Spermagonia epiphyllous, in groups, globoid, cinnamon-brown, 100–150 μ in diameter. Aecia and uredia lacking. Telia amphigenous, chiefly hypophyllous, in groups accompanying the spermagonia, round, compact, cinnamon-brown, becoming cinereous from germination, 0.3–1.0 mm. in diameter; teliospores narrowly ellipsoid, subclavate, or broadly fusiform, 20–30 × 50–90 μ, rounded or somewhat narrowed at both ends, constricted at the septum, germinating without a resting period; wall pale cinnamon or yellowish, smooth, 1–2 μ in thickness, thickened apically to 3–4 μ; pore of upper cell apical, of lower next to the septum; pedicel hyaline, thin-walled, persistent, 12–23 μ in width, 160–200(–250) μ long.

HOSTS AND DISTRIBUTION: *Salvia* sp., Ecuador.

TYPE LOCALITY: Huigra, Ecuador, on *Salvia* sp.

ILLUSTRATIONS: FIG. 1 (from type).

SPECIMENS EXAMINED: On *Salvia* sp.: Huigra, Ecuador, Aug. 4, 1920, Holway *842 A* (type).

Puccinia astricta, known only from the type locality, is distinct from the other rusts treated herein, being the only lepto-species in the group. The long, stout teliospore pedicels are characteristic of *P. astricta* and *P. roesteliiformis.*

2. PUCCINIA ROESTELIIFORMIS Lagerh. apud Sydow, Monogr. Ured. **1**: 292. 1904.

Spermagonia epiphyllous, in groups, subepidermal, globoid, cinnamon-brown or yellowish, 130–170 μ in diameter. Aecia hypophyllous, roestelioid, 0.3–1.0 mm. in diameter, 1–4 mm. high, scattered or in groups up to 5 mm. across; peridial cells angularly elliptical, hexagonal or rhomboidal, hyaline, 24–26 × 32–48 μ in surface view, inner wall 6–10 μ, coarsely verrucose, outer wall 3–6 μ, striate; aeciospores broadly ellipsoid, globoid, or pyriform, 23–30 × 30–43 μ; wall pale yellow, 3–5(–7) μ in thickness, tessellate. Uredia hypophyllous, scattered, round, compact, paraphysate, pale cinnamon-brown, 0.2–1.0 mm. in diameter; urediospores mostly globoid, 20–28 × 20–28 μ, occasionally broadly ellipsoid, 20–25 × 23–30 μ; wall pale cinnamon or yellowish, closely and finely punctate-verrucose, 1.5–2.5 μ thick, pores 6–9, scattered, located in slightly concave areas of the wall, each covered by a conspicuous hyaline papilla 1–2 μ thick; uredial paraphyses hyaline, thin-walled, cylindrical or clavate, 13–26 μ broad at the apex, up to 135 μ long. Telia hypophyllous, scattered, round, compact, becoming gelatinous when moist, cinnamon-brown, 0.2–1.5 mm. in diameter; teliospores cylindrical, (13–)16–23(–27) × (80–)100–155(–170) μ, rounded

above, obtuse or slightly rounded below, slightly or not constricted at the septum, germinating without a resting period; wall cinnamon-brown, paler over the pores, smooth, 1–2 μ, thickened to 3–4 μ at the angles of the septum and at the apex; pore of upper cell apical, of lower next to the septum; pedicel hyaline, thin-walled, persistent, 13–23 μ in width, 250–300(–330) μ long.

HOSTS AND DISTRIBUTION: *Salvia corrugata* Vahl, Ecuador; *Salvia* sp., Ecuador.

TYPE LOCALITY: Guayras-caja, Ecuador, on *Salvia* sp.

ILLUSTRATIONS: Sydow, Monogr. Ured. 1: Tab. XIX, FIG. 275; this paper, FIG. 2 (from type).

EXSICCATI: Reliq. Holw. *429*.

SPECIMENS EXAMINED: On *Salvia corrugata:* Biblian, Prov. Canar, Ecuador, Sept. 9, 1920, Holway *966:* Ambato, Dept. Tunguragua, Ecuador, March, 1919, Pachano *192;* Guaranda, Prov. Bolivar, Ecuador, April 26, 1942, Haught *3281*. On *Salvia* sp.: Guayras-caja, Prov. Chimborazo, Ecuador, Sept., 1891, G. Lagerheim (type).

This species differs strikingly from other *Salvia* rusts in several respects, the chief points of difference being the long, cylindrical teliospores, the tremelloid nature of the telia, and the presence of paraphyses in the uredia. The urediospores are distinct from those of the other South American species in having 6–9 scattered pores. In this and in shape and wall characters the urediospores of *P. roesteliiformis* show a marked resemblance to those of *P. bithynica* Magn., a species occurring on *Salvia* in the Mediterranean region.

The telia of *P. roesteliiformis* become somewhat gelatinous when moist, resembling those of species of *Gymnosporangium* in this respect, as Sydow (8) has pointed out. The roestelioid nature of the aecia, upon which the species name is based, is another point of similarity. However, the host relationship of *P. roesteliiformis* and the presence of paraphysate uredia in this species would seem to leave little doubt that Lagerheim was correct in naming it as a species of *Puccinia* rather than placing it in *Gymnosporangium.*

The aecia and aeciospores of *P. roesteliiformis* are much like those of *P. gilliesii* Speg. and *P. sphacelicola* Jacks. & Holw.; the aeciospores differ, however, in lacking the papilla or beak characteristic of the latter two species. Jackson (5) has described the

aeciospore wall in *P. roestcliiformis* as "reticulate"; actually it is tessellate, with large, flat, closely-fitting tubercles.

3. PUCCINIA ALBICERA Jacks. & Holw. Mycologia **24**: 73. 1932.

Spermagonia epiphyllous, in groups, subepidermal, globoid, chestnut-brown, 100–180 μ in diameter. Aecia amphigenous, chiefly epiphyllous, aecidioid, in groups up to 2 mm. across; peridial cells oblong or elliptical, hyaline, 16–30 × 30–40 μ in surface view, inner wall 2–3 μ, punctate-verrucose, outer wall 10–13 μ, finely striate; aeciospores globoid, ellipsoid, or oblong, 16–26 × 16–33 (–43) μ, wall yellowish, 1–2 μ thick, coarsely verrucose, with square, flat tubercles. Uredia hypophyllous, scattered, round, pulverulent, cinnamon-brown or yellowish, 0.1–0.5 mm. in diameter; urediospores oblate-spheroid, 20–25 μ broad by 17–23 μ high; wall cinnamon-brown, echinulate, 1–1.5 μ thick, pores 1 or 2, about ½ subequatorial. Telia hypophyllous, scattered, round, compact, cinnamon- or chestnut-brown, becoming cinereous from germination, 0.2–1.0 mm. in diameter; teliospores clavate, cylindrical or narrowly ellipsoid (13–)16–23 × (43–)50–56(–66) μ, rounded or truncate above, somewhat narrowed below, sharply constricted at the septum, germinating without a resting period; wall cinnamon-brown, smooth, 1–1.5 μ thick, pore of upper cell apical, of lower at the septum, each covered by a hyaline umbo 3–6 μ thick; pedicel hyaline, thin-walled, fragile, frequently breaking away completely, occasionally persisting and up to 80 μ long.

HOSTS AND DISTRIBUTION: *Salvia derasa* Benth., Ecuador; *Salvia tortuosa* Kunth, Ecuador.

TYPE LOCALITY: Quito, Ecuador, on *Salvia derasa.*

ILLUSTRATIONS: FIG. 3 (from type).

SPECIMENS EXAMINED: On *Salvia derasa:* Quito, Ecuador, Aug. 14, 1920, Holway *889* (type). On *Salvia tortuosa:* Quito, Ecuador, Aug. 21, 1920, Holway *930;* Pichincha, Ecuador, Dec. 29, 1938, Balls *5819.*

Puccinia albicera shows some resemblance to *P. soledadensis* Mayor, but differs from that species in having narrower teliospores and smaller urediospores. An additional point of difference in teliospore characteristics is in the nature of the apical umbo, which is smooth in *P. albicera,* but striate in *P. soledadensis.*

4. PUCCINIA SOLEDADENSIS Mayor, Mem. Soc. Neuch. Sci. Nat. **5**: 494. 1913.

Uredo archeriana Kern, Thurston & Whetzel, Mycologia **25**: 485. 1933.

Spermagonia and aecia unknown. Uredia hypophyllous, scattered, round, pulverulent, chestnut-brown, 0.2–1.0 mm. in diameter; urediospores variable, oblate-spheroid and 23–28 μ broad by 20–25 μ high, obovoid, 20–25 × 21–33 μ, or ellipsoid, 20–28 × 25–40(–43) μ; wall usually dark cinnamon-brown, occasionally paler, sparsely and prominently echinulate, with conspicuous conical spines, 1.5–2 μ in thickness, pores 2, rarely 3, equatorial or ⅓–½ subequatorial. Telia hypophyllous, scattered, round, compact, pulvinate, chestnut-brown, 0.3–1.5 mm. in diameter; teliospores narrowly ellipsoid, oblong, or subclavate, 20–30 × 43–56 μ, rounded above, rounded or narrowed below, constricted at the septum, germinating without a resting period; wall cinnamon-brown, smooth, 1–1.5 μ thick; pore of upper cell apical, capped by a striate, hyaline or yellowish umbo 4–7 μ thick, pore of lower cell next to the septum, covered by a hyaline or yellowish umbo 3–4 μ thick; pedicel hyaline, thin-walled, persistent, usually equal to the spore in length, occasionally up to 70 μ long.

HOSTS AND DISTRIBUTION: *Salvia angulata* Benth., Venezuela; *Salvia bogotensis* Benth., Colombia; *Salvia calocalicina* Briq., Colombia; *Salvia latens* Benth., Colombia; *Salvia* sp., Colombia.

TYPE LOCALITY: Soledad, Colombia, on *Salvia* sp.

ILLUSTRATIONS: Mayor, Mem. Soc. Neuch. Sci. Nat. **5**: FIG. 26; this paper, FIG. 4 (from type).

SPECIMENS EXAMINED: On *Salvia angulata:* Merida, Venezuela, Jan. 20, 1928, Pittier *12642*. On *Salvia bogotensis:* Soata, Dept. Boyaca, Colombia, Sept. 6, 1938, Cuatrecasas *1006*. On *Salvia calocalicina:* Salento, Dept. Caldas, Colombia, July 25–31, 1922, Pennell *8901*. On *Salvia latens:* Sta. Elena, Colombia, Dec. 28, 1930, Archer *H-201* (type of *Uredo archeriana*). On *Salvia* sp.: Soledad, Colombia, Oct. 6, 1910, Mayor *79* (type).

Mayor (**7**) described this species from a specimen having only the telial stage, and pointed out that its development resembled that of a lepto-form. However, during the course of the present study the writer obtained specimens of *P. soledadensis* in which uredia as well as telia were found. These specimens are on leaves of *Salvia bogotensis* and *S. calocalicina* obtained from the phanerogamic collections of the U. S. National Herbarium.

The urediospores of *P. soledadensis* are variable in shape, ranging from oblate-spheroid to narrowly ellipsoid. The wall is characteristically dark cinnamon, with large, conical spines.

FIGS. 1–8. 1. Teliospore of *Puccinia astricta* (from type), × 800. 2. Teliospore of *P. roesteliiformis* (from type), × 800. 3. Teliospores of *P. albicera* (from type), × 800. 4. Teliospore of *P. soledadensis* (from type), × 800. 5. Teliospore of *P. conspersa* (from type), × 800. 6. Teliospore of *P. gilliesii* (from type). × 800. 7. Teliospores of *P. aenigmatica* (from type), × 800.

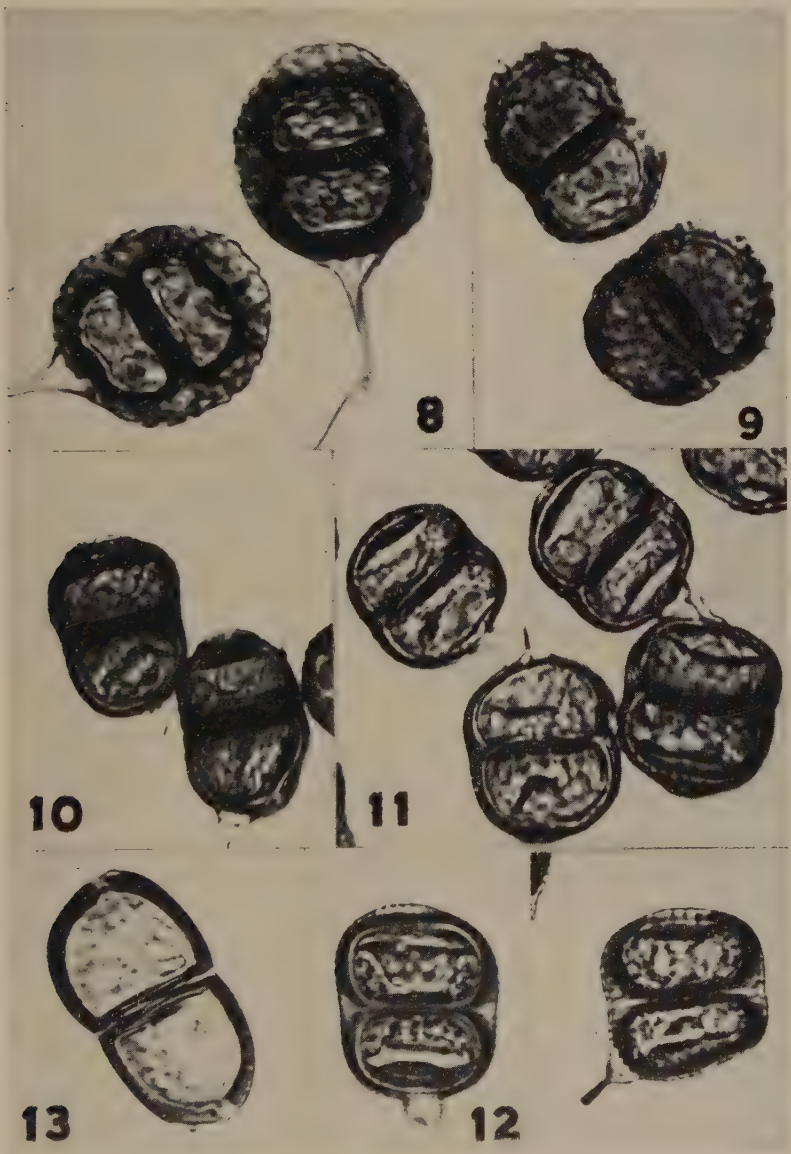
An examination of the type specimen of *Uredo archeriana* during this study revealed the presence of a sorus bearing teliospores of *P. soledadensis*. The urediospores in this specimen are larger and more narrowly ellipsoid than those of the other specimens of *P. soledadensis*, but agree well in wall color and type of echinulation. In accord with these findings *Uredo archeriana* is reduced to synonymy with *P. soledadensis*.

5. PUCCINIA CONSPERSA Diet. Hedwigia **36**: 30. 1897.

Puccinia uliginosa Speg. Anal. Mus. Nac. Hist. Nat. Buenos Aires **23**: 26. 1912 (not *P. uliginosa* Juel 1894).

Spermagonia epiphyllous, in groups, subepidermal, globoid, cinnamon-brown, 85–165 μ in diameter. Aecia amphigenous, aecidioid, scattered or in groups up to 2 mm. across or up to 3 mm. in length along the veins; peridial cells elliptical or oblong, hyaline, 20–27 × 27–43 μ in surface view, inner wall 2–4 μ, coarsely verrucose, the tubercles forming conspicuous ridges, outer wall 6–10 μ, finely verrucose; aeciospores globoid or ellipsoid, occasionally angularly ellipsoid or oblong, 16–23 × 23–33 μ; wall pale yellow, 1–2 μ thick, finely verrucose. Uredia hypophyllous, scattered, round, pulverulent, cinnamon-brown, orange, or yellowish, 0.1–0.7 mm. in diameter; urediospores asymmetrical, broadly ellipsoid or obovoid with the pores in surface view, narrower and more nearly oblong with the pores in optical section, 16–23 × 20–26(–30) μ; wall pale cinnamon, echinulate, about 1 μ thick, pores 2, equatorial. Telia hypophyllous, occasionally also caulicolous, scattered or in groups up to 2 mm. across, round or elliptic, pulvinate, somewhat compact, chestnut-brown, 0.1–0.5 mm. in diameter on leaves, 0.5–1.5 mm. across and up to 3 mm. long on stems; teliospores ellipsoid or oblong, 17–23(–28) × 26–40(–43) μ, rounded at both ends, not or slightly constricted at the septum, germinating without a resting period; wall cinnamon-brown or yellowish, smooth below, verruculose or nearly smooth above, 1–1.5 μ thick, thickened to 2–3 μ at the angles of the septum, pore of upper cell apical, of lower next to the septum, each capped by a hyaline umbo 2–4 μ thick: pedicel hyaline, thin-walled, fragile, frequently breaking away completely, when present about ½ the length of the spore, occasionally up to 65 μ long.

HOSTS AND DISTRIBUTION: *Salvia arenaria* St. Hil., Brazil; *Salvia procurrens* Benth., Argentina; *Salvia sellowiana* Benth., Brazil; *Salvia serrata* Benth. (*Salvia pallida* Benth.), Argentina; *Salvia splendens* Sellow, Brazil, Argentina; *Salvia uliginosa* Benth., Argentina; *Salvia* sp., Brazil.



FIGS. 8–13. 8. Teliospores of *P. porphyretica* (from type), × 800. 9. Teliospores of *P. conturbata* (from type), × 800. 10. Teliospores of *P. mitoensis* (from type), × 800. 11. Teliospores of *P. sana* (from type), × 800. 12. Teliospores of *P. sphacelicola* (from type), × 800. 13. Teliospore of *P. striatifera* (from type), × 800.

TYPE LOCALITY: Serra Geral, Brazil, on *Salvia* sp.

ILLUSTRATIONS: Sydow, Monogr. Ured. **1**: Tab. XIX, FIG. 273; this paper, FIG. 5 (from type).

EXSICCATI: Reliq. Holw. *431, 433, 439.*

SPECIMENS EXAMINED: On *Salvia arenaria:* São Paulo, Brazil, May 2, 1922, Holway *1802.* On *Salvia procurrens:* La Plata, Argentina, March 28, 1947, Lindquist. On *Salvia sellowiana:* São Paulo, Brazil, May 6, 1922, Holway *1806.* On *Salvia serrata:* La Plata, Argentina Dec. 3, 1921, Lindquist. On *Salvia splendens:* Rio de Janeiro, Brazil, Aug. 15, 1921, Holway *1039;* La Plata, Argentina, April 17, 1924, Marchionatto *8352.* On *Salvia uliginosa:* Buenos Aires, Argentina, June 4, 1910, Buisel (type of *P. uliginosa* Speg.); La Plata, Argentina, April 12, 1947, Lindquist. On *Salvia* sp: Serra Geral, Brazil, April, 1891, Ule *1776* (type); Rio De Janeiro, Brazil, Nov. 8, 1921, Holway *1285, 1300;* São Paulo, Brazil, July 2, 1922, Holway *1993.*

The teliospores of *Puccinia conspersa* are somewhat like those of *P. soledadensis* in general appearance, but are much smaller. The urediospores are characteristically asymmetrical with the pores located in the flattened sides.

Examination of the type specimen of *P. uliginosa* Speg. shows that Jackson (**5**) was correct in listing this species as a synonym of *P. conspersa.*

PUCCINIA CONSPERSA var. **paramensis** stat. nov.

Puccinia paramensis Mayor, Mem. Soc. Neuch. Sci. Nat. **5**: 493. 1913.

A forma typica speciei differt teliosporis 16–22 × 22–30 μ; membrana uniformiter 1 μ crassa, ad apicem granulo-verrucosa.

Spermagonia and aecia unknown. Uredia, urediospores, and telia as in the typical form of *P. conspersa.* Teliospores broadly ellipsoid, 16–22 × 22–30 μ, rounded at both ends, slightly constricted at the septum, germinating without a resting period; wall about 1 μ thick, pale cinnamon or yellowish, granular-verrucose above, appearing smooth in the lower half, pore of upper cell apical, of lower next to the septum, each with a hyaline inconspicuous umbo 1–2.5 μ thick; pedicel hyaline, thin-walled, fragile, usually breaking away at the point of attachment.

On leaves of *Salvia carnea* Kunth in Colombia and on leaves of *Salvia killipiana* Epl. in Venezuela.

HOSTS AND DISTRIBUTION: *Salvia carnea* Kunth, Colombia; *Salvia killipiana* Epl., Venezuela.

TYPE LOCALITY: Paramos, Colombia, on *Salvia carnea.*

ILLUSTRATIONS: Mayor, Mem. Soc. Neuch. Sci. Nat. **5**: FIG. 25.

SPECIMENS EXAMINED: On *Salvia carnea:* Paramos, Colombia, Oct. 5, 1910, Mayor *83* (type). On *Salvia killipiana:* Lagua Negra, Merida, Venezuela, Feb. 27, 1931, Reed *839.*

In establishing *Puccinia paramensis,* Mayor (**7**) described only the telial stage. A close examination of his specimen during this study disclosed the presence of a few uredia bearing urediospores which agree well with those of *P. conspersa.* Later a specimen on *Salvia killipiana,* agreeing with Mayor's species and having abundant uredia and telia, was obtained from the phanerogamic collections of the U. S. National Herbarium. The teliospores in this specimen, although smaller and thinner-walled than those of *P. conspersa,* resemble the latter closely in the color and marking of the wall. It seems advisable on the basis of these observations to reduce *P. paramensis* to varietal rank.

6. PUCCINIA GILLIESII Speg. Anal. Soc. Ci. Argent. **47**: 265. 1899.

Puccinia obesa Sydow, Monogr. Ured. **1**: 289, 1904.

Spermagonia not seen. Aecia hypophyllous and caulicolous, short-cylindric, in groups up to 3 mm. across on leaves, up to 12 mm. long and causing hypertrophy and distortion on stems; peridial cells elliptical, angularly oval or oblong, pale yellow, 16–33 × 43–100 μ in surface view, inner wall 7–10 μ thick, verrucose, outer wall 5–9 μ thick, finely striate; aeciospores variable in shape, ellipsoid, pyriform, broadly fusiform, or oblong, 12–21 × 28–58 μ, wall pale yellow, verrucose-tesselate with large, flat tubercles, 3–4 μ thick at the sides, thickened to 5–7 μ at the ends, frequently prolonged at one end to form a conspicuous beak 7–14(–18) μ long. Uredia amphigenous, scattered, roundish, pulvinate, compact, light chestnut or cinnamon-brown, 0.5–4.0 mm. in diameter; urediospores broadly ellipsoid, obovoid or globoid, 23–30 × 27–37 μ; wall cinnamon-brown or yellowish, 4–6 μ thick, sparsely and prominently echinulate, with large conical spines, pores 3–4, equatorial, obscure. Telia amphigenous, scattered, roundish, pulverulent, blackish brown, 0.5–2.0 mm. in diameter; teliospores ellipsoid, 27–39 × 37–52 μ, rounded at both ends, not or slightly constricted

at the septum; wall 5–8 μ thick, thickened to 10–12 μ over the pores, obscurely and evenly verrucose with low, flat tubercles, laminate, the inner layer chocolate- or dark chestnut-brown, the outer cinnamon or yellowish, pore of upper cell apical, of lower about ⅔ of the distance between the septum and the pedicel, each pore covered by a yellowish, slightly umbonate thickening; pedicel brownish or yellow next to the spore, becoming hyaline downward, thick-walled, persistent, occasionally attached obliquely, 6–8 μ in width at the point of attachment, up to 150 μ long.

HOSTS AND DISTRIBUTION: *Salvia bangii* Rusby, Bolivia; *Salvia gilliesii* Benth., Argentina; *Salvia humboldtiana* Dietr., Ecuador.

TYPE LOCALITY: Cordoba, Argentina, on *Salvia gilliesii*.

ILLUSTRATIONS: Sydow, Monogr. Ured. 1: Tab. XIX, FIG. 272; this paper, FIG. 6 (from type).

SPECIMENS EXAMINED: On *Salvia bangii:* Cochabamba, Bolivia, Feb. 23, 1920, Holway *314*. On *Salvia gilliesii:* La Falda de la Punilla, Prov. Cordoba, Argentina, Sept. 12, 1896, Hieronymus (type of *P. obesa*); Cordoba, Argentina, May, 1899, Stuckert *6954* (type); Cacheuta, Argentina, March 9, 1909, Spegazzini: Prov. Salta, Argentina, April 8, 27, 1925, Venturi *3674, 3764;* San Rafael, Mendoza, Argentina, Nov. 30, 1940, Leal *6916;* Las Heras, Mendoza, Argentina, July 27, 1941, Leal *7645;* Las Heras, Mendoza, Argentina, May 7, 1944, Leal *9332*. On *Salvia humboldtiana:* Ambato, Prov. Tunguragua, Ecuador, July 9, 1939, Balls *7152*.

Puccinia gilliesii is readily distinguishable from other species on *Salvia* because of the thick-walled, prominently echinulate urediospores. The number of germ pores is somewhat variable, with 3- and 4-pored urediospores occurring in approximately equal numbers. The aecia and aeciospores of this species are much like those of *P. roesteliiformis* and *P. sphacelicola*. The aeciospore wall is distinctive, however, in being markedly thickened at the ends and frequently prolonged to form a conspicuous beak.

Although the teliospores of *P. gilliesii* resemble those of *P. aenigmatica* Jacks. & Holw. and *P. porphyretica* Jacks. & Holw. in having thick, bilaminate walls, they differ in the position of the pore in the lower cell and in the type of wall sculpturing.

7. PUCCINIA AENIGMATICA Jacks. & Holw. Mycologia 24: 72. 1932.

Spermagonia and aecia unknown. Uredia amphigenous, scattered, round, pulverulent, chestnut-brown. 0.2–0.5 mm. in diameter; urediospores oblate-spheroid, 23–30 μ broad by 20–23 μ high; wall dark cinnamon-brown, echinulate, 1–1.5 μ thick, pores 2, rarely 3 or 4, usually near the hilum, occasionally ⅓–½ subequatorial. Telia amphigenous, scattered, round, pulverulent, blackish brown, 0.3–1.0 mm. in diameter; teliospores broadly ellipsoid or globoid, 30–38 × 33–46 μ, rounded at both ends, not constricted at the septum; wall laminate, chestnut-brown, coarsely verrucose, 5–8 μ thick, thickened to 10–14 μ and paler over the pores, pore of upper cell apical, of lower near the septum; pedicel hyaline, thick-walled, persistent, 8–11 μ wide at the point of attachment, up to 150 μ long.

HOSTS AND DISTRIBUTION: *Salvia bangii* Rusby (*Salvia erythradena* Briq.), Bolivia; *Salvia haenkei* Benth., Bolivia; *Salvia orbignaei* Benth. (*Salvia kuntzeana* Briq.), Bolivia; *Salvia sarmentosa* Epl., Peru.

TYPE LOCALITY: Cochabamba, Bolivia, on *Salvia orbignaei* (*Salvia kuntzeana*).

ILLUSTRATIONS: FIG. 7 (from type).

EXSICCATI: Reliq. Holw. *417, 418* (the latter as *Puccinia mitrata* Syd.).

SPECIMENS EXAMINED: On *Salvia bangii:* Cochabamba, Bolivia, March 10, 1920, Holway *386*. On *Salvia haenkei:* Cochabamba, Bolivia, June, 1947, Cardenas *225*. On *Salvia orbignaei: Cochabamba,* Bolivia, March 1, 1920, Holway *358* (type). On *Salvia sarmentosa:* Ollantaytambo, Peru, April 26, 1915, Cook & Gilbert *375*.

One of the specimens cited above, Cardenas' No. *225* on *S. haenkei,* has urediospores which range in shape from globoid to broadly ellipsoid and thus do not agree completely with those of the type. However, the position of the pores and the thickness of the wall, as well as the size of uredia, indicate that this specimen should be referred to *P. aenigmatica.*

Holway's specimen on *S. bangii* (*S. erythradena*) was originally determined as *P. mitrata* by Jackson (**5**). Although the urediospores in this specimen approach those of *P. mitrata* in size, in all

other respects the specimen agrees well with *P. aenigmatica* and is accordingly referred to that species.

8. PUCCINIA PORPHYRETICA Jacks. & Holw. Mycologia 24: 76, 1932.

Spermagonia and aecia unknown. Uredia amphigenous, scattered, round, pulverulent, chestnut-brown, 0.4–3.0 mm. in diameter; urediospores broadly ellipsoid or obovoid, 20–26 × 24–34 μ; wall cinnamon-brown, prominently echinulate, about 3 μ thick, pores 2, equatorial. Telia amphigenous, scattered, round, pulverulent; chocolate-brown, 0.3–5.0 mm. in diameter; teliospores broadly ellipsoid or globoid, 26–38 × 30–46 μ, rounded at both ends, not constricted at the septum; wall distinctly laminate, uniformly light chestnut- or cinnamon-brown, occasionally dark chestnut-brown, coarsely verrucose, 5–8 μ thick, thickened to 9–14 μ and paler over the pores, pore of upper cell apical, of lower next to the septum; pedicel hyaline, thick-walled, persistent, 6–10 μ in width at the point of attachment, up to 130 μ long.

HOSTS AND DISTRIBUTION: *Salvia haenkei* Benth. (*Salvia bridgesii* Britton, *Salvia pseudoavicularis* Briq.), Bolivia; *Salvia* sp., Peru.

TYPE LOCALITY: Cuzco, Peru, on *Salvia* sp.

ILLUSTRATIONS: FIG. 8 (from type).

EXSICCATI: Reliq. Holw. *416, 419, 425.*

SPECIMENS EXAMINED: On *Salvia haenkei:* Cochabamba, Bolivia, Feb. 26, 1920, Holway *329;* Sorata, Bolivia, April 15, 1920, Holway *527.* On *Salvia* sp.: Cuzco, Peru, July 4, 1920, Holway *761* (type).

Puccinia porphyretica and *Puccinia aenigmatica* are representatives of a group of New World *Salvia* rusts which resemble one another closely with respect to teliospore characters. The other species in this group are *Puccinia mitrata* Syd. and *Puccinia gentilis Arth.,* known to occur only in North America (2). All have broad teliospores with thick, coarsely verrucose, bilaminate walls. These rusts can be separated on the basis of urediospore characters, size of uredia, and the nature of the teliospore pedicel. The chief points of distinction are given in the following key:

Teliospore pedicel thin-walled, 70–100 μ long.
Urediospores 16–21 × 20–23 μ.....*P. mitrata.*
Urediospores 20–23 × 23–29 μ.....*P. gentilis.*

Teliospore pedicel thick-walled, 100–150 μ long.
Urediospore wall 1–1.5 μ thick, uredia 0.2–0.5 mm. in diameter.....*P. aenigmatica.*
Urediospore wall 3 μ thick, uredia 0.4–3.0 mm. in diameter.....*P. porphyretica.*

9. PUCCINIA CONTURBATA Jacks. & Holw. Mycologia **24**: 75. 1932.

Spermagonia and aecia unknown. Uredia hypophyllous, scattered, round, pulverulent, cinnamon-brown, 0.2–0.5 mm. in diameter; urediospores oblate-spheroid, 23–26 μ broad by 20–22 μ high, or obovoid, 18–24 × 22–25 μ; wall cinnamon-brown, closely echinulate, 1–1.5 μ thick in the upper two-thirds, becoming 2–2.5(–3) μ thick and darker in the lower third, pores 2, occasionally 3, about ½ subequatorial. Telia hypophyllous, scattered, round, pulverulent, blackish brown, 0.1–0.5 mm. in diameter; teliospores ellipsoid or oblong, 23–26 × 30–36(–40) μ, rounded at both ends, constricted at the septum; wall dark chestnut-brown, rugose-reticulate, 2–3 μ in thickness, thickened to 4–6 μ and paler at the apex, pore of upper cell apical, of lower next to the septum; pedical hyaline, thin-walled, fragile, usually breaking away at the point of attachment.

HOSTS AND DISTRIBUTION: *Salvia quitensis* Benth., Ecuador; *Salvia* sp., Ecuador.

TYPE LOCALITY: Huigra, Ecuador, on *Salvia* sp.

ILLUSTRATIONS: FIG. 9 (from type).

SPECIMENS EXAMINED: On *Salvia quitensis:* Cuenca, Ecuador, Sept. 15, 1920, Holway *992*. On *Salvia* sp.: Huigra, Ecuador, Aug. 4, 1920, Holway *842* (type).

The teliospores of this species show some resemblance to those of a North American species, *Puccinia diutina* Mains & Holw. The chief point of distinction between the two species is in the nature of the urediospore wall. In *P. conturbata* the wall is thin in the upper two-thirds, but markedly thickened basally, while the condition in *P. diutina* is the reverse of this. An additional point of difference is in the position of the urediospore pores, which in *P. diutina* are basal next to the hilum.

10. **Puccinia mitoensis** sp. nov.

Spermagoniis et aeciis adhuc ignotis. Urediis hypophyllis, sparsis, rotundatis, pulverulentis, cinnamomeo-brunneis, 0.2–0.5 mm. diam.; urediosporis late globoideis, 23–30 μ latis, 20–26 μ altis; membrana cinnamomeo-brunnea, moderate echinulata, 1.5–2 μ crassa, poris germ. 2 vel 3, subaequatorialibus vel aequatoriaibus. Teliis hypophyllis, sparsis, rotundatis, pulverulentis,

atro-brunneis, 0.1–0.5 mm. diam.; teliosporis ellipsoideis vel oblongis, 18–24 × 26–32 μ, utrinque rotundatis, medio non vel leniter constrictis; membrana castaneo-brunnea, 1.5–2.5 μ crassa, supra poros usque ad 4 μ in umbonem pallidiorem incrassata, obscure rugoso-reticulata; poro superiore apicali, inferiore juxta pedicellum sito; pedicello hyalino, fragili, brevissimo.

Spermagonia and aecia unknown. Uredia hypophyllous, scattered, round, pulverulent, cinnamon-brown, 0.2–0.5 mm. in diameter; urediospores oblate-spheroid, 23–30 μ broad by 20–26 μ high; wall cinnamon-brown, moderately echinulate, 1.5–2 μ thick, pores 2, occasionally 3, about ½ subequatorial or occasionally equatorial. Telia hypophyllous, scattered, round, pulverulent, blackish brown, 0.1–0.5 mm. in diameter; teliospores ellipsoid or oblong, 18–24 × 26–30 μ, rounded at both ends, not or slightly constricted at the septum; wall chestnut-brown, obscurely rugose-reticulate, 1.5–2.5 μ in thickness, thickened to 3–4 μ at the angles of the septum, pore of upper cell apical, of lower next to the pedicel, each capped by a flat yellowish umbo about 2 μ thick; pedicel hyaline, thin-walled, fragile, usually breaking away at the point of attachment.

On leaves of *Salvia punctata* var. *glabra* in Peru.

HOSTS AND DISTRIBUTION: *Salvia punctata* R. & P. var. *glabra* Epl., Peru.

TYPE LOCALITY: Mito, Peru, on *Salvia punctata* var. *glabra*.

ILLUSTRATIONS: FIG. 10 (from type).

SPECIMEN EXAMINED: On *Salvia punctata* var. *glabra:* Mito, Peru, July 8, 22, 1922, Macbride & Featherstone.

Puccinia mitoensis is much like *P. conturbata,* but differs in having smaller, thinner-walled teliospores in which the pore of the lower cell is located next to the pedicel. The finer, less conspicuous marking of the teliospore wall in *P. mitoensis* serves further in distinguishing the two species.

11. PUCCINIA SANA Jacks. & Holw. Mycologia **24**: 77. 1932.

Spermagonia and aecia present but too old for accurate description. Uredia hypophyllous, scattered, round, pulverulent, cinnamon-brown, 0.1–0.5 mm. in diameter; urediospores oblate-spheroid, 23–26 μ broad by 20–23 μ high, or occasionally obovoid or ellipsoid, 20–23 × 23–26 μ; wall connamon-brown, moderately echinulate, 1–1.5 μ thick, pore 1, equatorial. Telia hypophyllous, scattered, round, pulverulent, chestnut- or chocolate-brown, 0.1–0.5 mm. in diameter; teliospores broadly ellipsoid or oblong, 20–27 × 26–36 μ, rounded at both ends, not or slightly constricted at the septum; wall light chestnut-brown or cinnamon-brown, finely and evenly

verrucose, 1.5–2 μ thick, thickened to 3 μ at the angles of the septum, pore of upper cell apical, of lower next to the septum, each capped by an inconspicuous hyaline umbo 1–2.5 μ thick; pedicel hyaline, thin-walled, fragile, usually broken off at or near the spore, occasionally up to 40 μ long.

HOSTS AND DISTRIBUTION: *Salvia leucocephala* Kunth, Ecuador; *Salvia* sp., Ecuador.

TYPE LOCALITY: Huigra, Ecuador, on *Salvia leucocephala.*

ILLUSTRATIONS: FIG. 11 (from type).

SPECIMENS EXAMINED: On *Salvia leucocephala* Kunth: Huigra, Ecuador, Aug. 4, 1920, Holway *835* (type); Huigra, Ecuador, Aug. 4, 1920, Holway *839*. On *Salvia* sp.: Chimborazo, Ecuador, Sept., 1891, Lagerheim.

Jackson (**5**) pointed out that *Puccinia sana* appeared to be much the same as an undescribed collection from Ecuador under the name *Puccinia andicola* Lagerh. The writer has examined a portion of Lagerheim's collection and has found that it is a specimen of *P. sana*. Although a few aecia are present they are too old to permit accurate description.

12. PUCCINIA SPHACELICOLA Jacks. & Holw. Mycologia **24**: 67. 1932.

Spermagonia amphigenous, in groups, subepidermal, globoid, chestnut-brown, 100–180 μ in diameter. Aecia amphigenous, chiefly hypophyllous, cylindric, 0.5–1.0 mm. in diameter and 1–3 mm. high, in groups up to 5 mm. long on leaf veins; peridial cells angularly oval or elliptical, yellowish, 30–40 × 34–50 μ in surface view, inner wall 9–12 μ thick, verrucose, outer wall 7–9 μ thick, finely striate; aeciospores globoid, ellipsoid, pyriform, or broadly fusiform, 23–32 × 30–42 μ; wall pale yellow or hyaline, verrucose-tessellate, 3–4 μ thick, occasionally prolonged at one end to form a papilla or short beak. Uredia unknown, probably lacking. Telia epiphyllous, scattered, round, compact, becoming pulverulent, chestnut-brown, 0.5–1.0 mm. in diameter; teliospores ellipsoid or oblong, 23–30 × 28–37 μ, rounded at both ends, not constricted at the septum; wall cinnamon-brown, paler over the pores, finely and evenly punctate-verrucose, appearing striate, about 2 μ thick, thickened to 3–4 μ over the pores and to 4–5 μ at the septum angles, pore of upper cell apical, of lower next to the pedicel; pedicel hyaline, thin-walled, fragile, breaking at or near the point of attachment, occasionally attached obliquely.

HOSTS AND DISTRIBUTION: *Salvia rumicifolia* Kunth, Ecuador.

ILLUSTRATIONS: FIG. 12 (from type).

EXSICCATI: Reliq. Holw. 426 (host listed as *Sphacele paniculata* Benth.).

Specimens examined: On *Salvia rumicifolia:* Riobamba, Ecuador, Aug. 10, 1920, Holway *865* (type); Quito, Ecuador, Aug. 23, 1920, Holway *937;* Riobamba, Ecuador, Feb. 4, 1934, Schimpff *933*.

Jackson (5) described *Puccinia sphacelicola* from Holway's No. *865*, collected at Riobamba, Ecuador. At that time the host was determined as *Sphacele paniculata* Benth. Later, Epling (4) cited Holway's No. *865* as a specimen of *Salvia rumicifolia*. The specimen cited above as Schimpff *933* is on leaves of *Salvia rumicifolia*, from the phanerogamic collections of the U. S. National Herbarium. These leaves were found to bear a few aecia and telia of *P. sphacelicola*.

13. **Puccinia striatifera** sp. nov.

Spermagoniis, aeciis et urediis adhuc ignotis. Teliis amphigenis, rotundatis, pulverulentis, castaneo-brunneis, sparsis, 0.2–1.5 mm. diam., vel in greges usque ad 4 mm. diam.; teliosporis ellipsoideis, 20–26 × 36–43 (–52) μ, utrinque rotundatis, medio constrictis; membrana cinnamomeo-brunnea, uniformiter 2 μ crassa, supra poros usque ad 3 μ leniter incrassata, striata, poro superiore apicali, inferiore juxta pedicellum sito; pedicello hyalino, fragili, brevissimo.

Spermagonia, aecia and uredia unknown. Telia amphigenous, round, compact, becoming pulverulent, chestnut-brown, scattered, 0.2–1.5 mm. in diameter or in groups up to 4 mm. across; teliospores ellipsoid, 20–26 × 36–43 (–52) μ, rounded at both ends, constricted at the septum; wall cinnamon-brown, uniform, about 2 μ thick, thickened slightly to about 3 μ over the pores, transversely striate; pore of upper cell apical, of lower next to the pedicel; pedicel hyaline, thin-walled, fragile, breaking away at the point of attachment. On leaves of *Salvia revoluta* in Peru.

HOSTS AND DISTRIBUTION: *Salvia revoluta* R. & P., Peru.

TYPE LOCALITY: Huariaca, Peru, on *Salvia revoluta*.

ILLUSTRATIONS: FIG. 13 (from type).

SPECIMENS EXAMINED: On *Salvia revoluta:* Huariaca, Peru, Sept. 13, 1922, Macbride & Featherstone *2407*.

This species, of which only the telial stage is known, is based on a specimen obtained from the phanerogamic collections in the her-

barium of the Chicago Museum of Natural History. It resembles *P. sphacelicola* in the marking of the teliospore wall and the position of the pore in the lower cell but differs in having longer teliospores which are constricted at the septum. The teliospore wall in *P. striatifera* is also much more uniform in thickness, lacking the thickening at the angles of the septum.

14. PUCCINIA IMPEDITA Mains & Holw. apud Arthur Mycologia **10**: 135. 1918.

Uredo salviarum Mayor, Mem. Soc. Neuch. Sci. Nat. **5**: 592. 1913.

Bullaria impedita Arth. & Mains, N. Am. Flora **7**: 493. 1922.

SPECIMENS EXAMINED: On *Salvia cataractarum* Briq.: Andes Orientales, Dept. Cundinamarca, Colombia, Oct. 12, 1910, Mayor *49*. On *Salvia macrostachya* Kunth: Pangor, Ecuador, Sept., 1891, Lagerheim. On *Salvia mayorii* Briq.: Andes Orientales, Dept. Cundinamarca, Colombia, Oct. 12, 1910, Mayor *47*. On *Salvia occidentalis* Sw.: Trinidad, March 3, 1921, Seaver; Moruga, Trinidad, March 19, 1921, Seaver; Huanuco, Peru, Sept. 23, 1922, Macbride & Featherstone. On *Salvia petiolaris* Kunth: Andes Centrales, Dept. Antioquia, Aug., Sept., 1910, Mayor *203* (type of *Uredo salviarum*). On *Salvia tiliaefolia* Vahl: Villa Aspiazu, Sur Yungas, Bolivia, May 28, 1920, Holway *675;* Hacienda Anacuri, Nor Yungas, Bolivia, June 2, 1920, Holway *700* (Reliq. Holw. *423*).

This species and the one immediately following occur chiefly in North America. Descriptions of them can be found in the paper by Baxter and Cummins (**2**) on the North American species of this group.

Holway's specimen No. *700* has telia as well as uredia. The teliospores are paler and thinner-walled than are those of North American specimens of this rust, but agree otherwise as do the urediospores.

Uredo salviarum Mayor was placed in synonymy with *Puccinia salviicola* Diet. & Holw. by Arthur (**1**). Later it was transferred to synonymy with *P. ballotaeflorae* Long by Kern, Thurston and Whetzel (**6**), on the basis of the number and position of the germ pores. The writer has examined Mayor's specimens of *U. sal-*

viarum and has found that this species agrees more closely with *P. impedita.* It has accordingly been transferred to synonymy with the latter species.

15. PUCCINIA FARINACEA Long, Bull. Torrey Club **29**: 115. 1902.
Puccinia prospera Arth. Bull. Torrey Club **46**: 118. 1919.
Dicaeoma prosperum Arth. N. Am. Flora **7**: 414. 1921.
Dicaeoma farinaceum Arth. N. Am. Flora *7*: 411. 1921.

SPECIMENS EXAMINED: On *Salvia rhinosina* Griseb.: Jujuy, Argentina, Feb. 15, 1939, Balls *6067.* On *Salvia stachydifolia* Benth.: Cagunilla, Bolivia, March, 1933, Cardenas *439.* On *Salvia* sp.: Vespucio, Dept. Oran, Argentina, July 14, 1937, Cabrera *4186.*

This highly variable species is widely distributed in North America, where it occurs from the central United States southward through Central America. The known range is here extended to include Bolivia and Argentina.

The collection on *Salvia* sp. from Argentina, Cabrera's No. *4186,* exhibits a phenomenon observed previously (2) in a specimen of *P. farinacea* from Guatemala. In addition to the typical overwintering spores the telia contain numerous pale, thin-walled teliospores which germinate without a resting period. The lability exhibited by such collections may be an indication that *P. farinacea* is tending toward the microcyclic condition.

EXCLUDED SPECIES

Puccinia griseola Lagerh, apud Syd., Monogr. Ured. **1**: 296. 1904.

This species has sessile teliospores and belongs in the genus *Polioma,* which has recently been reinstated by Baxter and Cummins (3) to accommodate four species having 2-celled sessile teliospores.

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GERTRUDE SIMMONS BURLINGHAM
1872–1952

Fred J. Seaver

(with portrait)

Dr. Gertrude S. Burlingham, well-known mycologist, passed away January 11, 1952 in her home in Winter Park, Florida, after a rather extended illness. Miss Burlingham was born in Mexico, New York, April 21, 1872, and spent all of her professional life in her native state. For many years she was a teacher of biology and grade adviser at Eastern District High School, Brooklyn. Early in her career, however, she adopted Vermont as her second home and throughout her professional life she spent her summers in her adopted state.

My first contact with Dr. Burlingham was while at the New York Botanical Garden, when on week ends this modest, quiet little woman used to wander into the herbarium and browse through the collections checking up the material collected by her during her summer vacation. How well do I recall how she showed with great pride the picture of the house she had built for herself on Newfane Hill, Vermont, in 1914. Even after her retirement to Florida in 1934 she continued to migrate with the birds to the old nesting place returning at first from California and later from Florida, where she purchased a winter home in 1936. At her request her physical remains were interred on the Hill she loved so much during her lifetime. But scientists, like "old soldiers," never die. They merely pass on but continue to live through the contributions they have left behind.

Dr. Burlingham was an outstanding authority on the genera *Russula* and *Lactarius*. Her interest, however, was not confined to these forms. She had a wide knowledge of the fungi in general and having grown up on a farm she was an all-round naturalist. In her zeal for field knowledge of the fungi she spent three seasons collecting on the Pacific coast, in Washington, Oregon and

California, and made a journey to Sweden to collect with the Swedish students of the Hymenomycetes, Lars Romell and Seth Lundell, and the Danish master, Jacob Lange. This, of course, was in addition to her constant field work along the Atlantic coast. She was a graduate of Syracuse University and took her doctor's degree in Columbia University in 1908. Much of her

GERTRUDE SIMMONS BURLINGHAM
Photograph by Tooley-Myron Studios

early work was done at the New York Botanical Garden and it is therefore quite appropriate that her extensive collections should be left to that institution, as has been done. A list of her publications, prepared by Dr. H. W. Rickett of the New York Botanical Garden follows:

Some Lactarii from Windham County, Vermont. Bull. Torrey Club **34**: 85–95. 9 Ap. 1907.

Suggestions for the study of the Lactariae. Torreya **7**: 118–123. 19 Je 1907.

A study of the influence of magnesium sulphate on the growth of seedlings. Jour. Am. Chem. Soc. **29**: 1095–1112. *f. 1–5.* Jl 1907.

A study of the Lactariae of the United States. Mem. Torrey Club **14**: 1–109. *f. 1–15.* 26 My 1908.

The Lactariae of North America—Fascicles I and II. Mycologia **2**: 27–36. 1 Ja 1910.

The Lactarieae of the Pacific Coast. Mycologia **5**: 305–311. 25 N 1913.

[Agaricaceae.] Tribe 2. Lactarieae. N. Am. Flora **9**: 172–200. 3 F 1910. 201–236. 30 Ap. 1915.

Methods for satisfactory field work in the genus *Russula*. Mycologia **9**: 243–247. 30 Jl 1917.

New species of *Russula* from Massachusetts. Mycologia **10**: 93–96. 4 Ap 1918.

A preliminary report on the *Russulae* of Long Island. Mem. Torrey Club **17**: 301–306. 10 Je 1918.

Some new species of *Russula*. Mycologia **13**: 129–134. *pl. 7* + *f. 1–6.* Je 1921.

Notes on species of *Russula*. Mycologia **16**: 16–23. *pl. 4, 5.* 19 Ja 1924.

Two new species of *Lactaria*. Mycologia **24**: 460–463. *f. 1–3.* 1 S 1932.

New or noteworthy species of *Russula* and *Lactaria*. Mycologia **28**: 263–267. *f. 1–8.* 1 Je 1936.

Two new species of *Russula* together with the spore ornamentation of some of our American russulas. Mycologia **31**: 490–498. *f. 1–14.* 1 Au 1939.

Interesting species of *Lactariae* from *Florida*. Mycologia **32**: 575–586. *f. 1–4.* 1 O 1940 (with Henry Curtis Beardslee).

Spore ornamentation of some American *Russulae* and a new species of *Lactaria*. Mycologia **34**: 8–12. *f. 1–14.* 1 F 1942.

Studies in North American Russulae. Mycologia **36**: 104–120. *f. 1, 2.* 1 F 1944.

Noteworthy species of *Lepiota* and *Lactaria*. Mycologia **37**: 53–64. *f. 1–7.* 3 F 1945.

Henry Curtis Beardslee. Mycologia **40**: 505, 506. *port.* 29 S 1948.

RENÉ VANDENDRIES
1874–1952

HAROLD J. BRODIE

(WITH PORTRAIT)

Mycologists in America join their colleagues in Europe and elsewhere in marking with sorrow the passing on March 28, 1952, of the renowned Belgian savant Dr. René Vandendries at his villa in Rixensart, Belgium.

In a modest laboratory attached to his home this man, single-handed, contributed a monumental volume to the knowledge of sexuality among basidiomycetous fungi. His name is associated forever with the names of Bensaude, Kniep, Buller and others who were responsible for revealing the unique characteristics of sexuality in the higher basidiomycetes. Vandendries' contribution to this "realm," as he called it, is two-fold: he analyzed the mating behavior of mycelia of many species and he carried out extensive and detailed studies of races of these fungi to determine the distribution of genes governing sexuality not only in large numbers of populations of European origin but also in populations from America.

Study of the subject of multiple-allelomorph heterothallism in the fungi as reviewed in 1949 by Whitehouse (see below), shows clearly that Vandendries' methodical research on fifty-five different species of Hymenomycetes was no mere cataloguing of their patterns of sexual behavior. From data of this kind Whitehouse and others have been able to present a remarkably full account of the distribution of homothallism and of bipolar and tetrapolar heterothallism among the Hymenomycetes and to state important generalizations as to the number of alleles at the loci for heterothallism in natural populations of the fungi. When it is realized that the analysis of each species or each race represents, on the average, the results of some three hundred pairings between single-spore

mycelia, it becomes evident that students of genetics and sexuality of the fungi owe much to Vandendries' work.

Vandendries was born in Louvain in 1874, the son of Flemish parents. At the University of Louvain he studied botany under the tutelage of Carnoy and there obtained the degree Doctor of

RENÉ VANDENDRIES AS A YOUNG MAN

Natural Sciences in 1898. Later he became a professor of the Athenée Royale of Antwerp and he taught also at Bruges and elsewhere. Upon retirement from university life in the late 1920's, Dr. Vandendries became an Inspector of those schools in Belgium which would be designated in America as normal schools or teachers' colleges. In whatever professional capacity he served, Vandendries was eminently successful: as a teacher he was lucid

in exposition and exacting in requirements; as an inspector he is remembered as a severe critic but a kindly judge.

Many honors were bestowed upon Dr. Vandendries by his country and by learned societies. Among these may be mentioned: Commander of the Order of Leopold, Grand Officer of the Order of the Crown of Belgium, Member of the Royal Thérésian Academy of Belgium and Member of the Royal Botanical Society of Belgium. The Botanical Society of America elected him Corresponding Member in 1947.

Dr. Vandendries married Anne Virginie Frélinckx who predeceased him in 1948. He is survived by one son, Henry, of the Evangelical Mission of Brussels, and a nephew, Dr. Corneille Vandendries, a chemist.

Prior to World War II, Vandendries went to live in Portugal because of his wife's poor health. There for several years the couple lived under conditions almost of privation owing to isolation from their native land and to the inaccessibility of their pension income. Scientific work was impossible for Vandendries during this time, but it was characteristic of him not to remain idle. Despite the burden of his wife's illness and his own failing strength, he began the writing of a long series of personal narratives under the title of "Contes et Souvenirs." Six volumes of these in longhand remain unpublished. The delightful stories are drawn from his own experiences and reveal his true character. The pages are filled with keen observations of nature, with fine humor and with a deep appreciation of beauty. He recounts his experiences at the International Genetics Congress in Ithaca which he attended by invitation in 1932; he reveals the pleasure derived from the visit to his laboratory of the late Professor T. H. Morgan; he tells amusing anecdotes about collecting and studying fungi. His stories of dogs and cats he has "known" remind one of La Fontaine, so beautifully and entertainingly are they written.

His students remember Vandendries as a short rather dapper man with an aristocratic bearing whose paramount personal characteristics were kindliness and good humor. To be received into his happy household not only as a student but almost as a son, was an experience of incalculable value. One was expected to put in long hours in the laboratory, but on Sundays there were walks

through the woods from Brussels to Wavre. On these occasions Vandendries would throw great energy and enthusiasm into hunting for fungi but would stop to examine and comment upon anything of special interest from geological formations to flowering plants and from Flemish pastry shops to gems of architecture. Despite his broad interests, his knowledge was amazingly profound. At other times there was music: Vandendries was an accomplished violist and Madame had at one time sung in the Brussels opera. His last admonition to the department student was usually: "No matter what you accomplish, your greatest reward will be the joy of the work itself."

Reference to over fifty of Vandendries' most important publications may be found in the articles which are listed below.

1. **Quitanilha, A.** 1933. Le problème de la sexualité chez les champignons. Bol. Soc. broteriana **8**: 1–100.
2. **Vandendries, R.** 1931. La contribution belge à l'étude du problème sexuel des champignons. Comptes Rendues Congr. Nat. des Sciences. Brussels 1930. 1–9.
3. **Whitehouse, H. L. K.** 1949. Multiple-allelomorph heterothallism in the fungi. New Phytol. **48**: 212–244.

DEPARTMENT OF BOTANY,
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NOTES AND BRIEF ARTICLES

Pigment Study of Dacrymyces Ellisii

Basidiospores of *Dacrymyces Ellisii* were germinated on malt agar. Bright yellow colonies 0.5–4 cm. in diameter resulted. After thirty days in the light at room temperature the colonies were manually removed from the agar. The collected colonies were homogenized and extracted with acetone. The acetone extract was evaporated, the pigments taken up in petroleum ether and, after saponification, chromatographed. Ten pigments were isolated.

TABLE I

a. Column 30 cm. long, 2 cm. diameter and packed with Alcoa activated alumina grade F-20. b. Absorption maxima in petroleum ether B. P. 30–60° C. c. Amounts of pigment present in 7 gms. dry weight of colonies after extraction with acetone. Amounts estimated from the absorption curves. d. All fractions give a positive test for carotenoids with concentrated sulfuric acid. e. Point of inflection. f. Tentatively identified from absorption spectra and color tests. g. Isolated from the hypophase, after saponification with 10% methanolic potassium hydroxide and hydrochloric acid, and chromatographed separately.

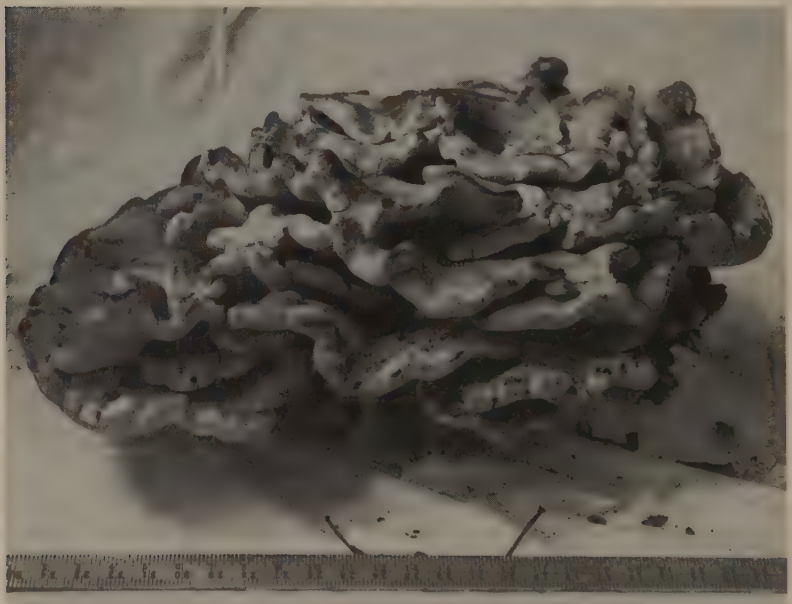
CAROTENOIDS FROM DACRYMYCES ELLISII

Band top down	Band color	Band width (cm.) [a]	Absorption maxima [b]			Estd. amts. (mg.) [c]	Pigment	Partition (%) [d] 95% CH_3OH	90%
1	YELLOW	0 - 0.2	424			0.01	I	0	0
2	ORANGE	0.6 - 0.8	400 [e]	444	477	0.1	II	10	0
3	PALE PEACH	1.0 - 2.8	420	450 [e]		0.014	III	30	0
4	DEEP PEACH	2.8 - 4.0	443			0.05	IV	15	0
5	PALE ROSE	4.2 - 8.2	424	448 [e]		0.005	V	-	-
6	PALE ROSE	8.8 - 23	446	473	501	0.009	LYCOPENE [f]	TRACE	0
7	LT YELLOW	24 - 26	430	463	495	0.025	γ-CAROTENE [f]	TRACE	TRACE
8	YELLOW	27 - 30 -	427 [e]	452	483	2.5	β-CAROTENE	TRACE	TRACE
9 [g]	ORANGE	1.2 - 1.4	-			0.015	H I	TRACE	0
10 [g]	YELLOW	1.4 - 3.0	-			0.004	H II	TRACE	0

By far the major pigment was β-carotene (TABLE I).—CALVIN HANNA AND THOMAS J. BULAT, Departments of Pharmacology and Botany, State University of Iowa.

A LARGE SPECIMEN OF POLYPORUS BERKELEYI

The extremely large size of the fruiting bodies produced by certain puffballs and polypores is always of interest. *Polyporus Berkeleyi* Fries is reputed to be the largest species of *Polyporus* found in the United States, and recently an unusually large fruc-



POLYPORUS BERKELEYI

tification of this species was collected by Mr. H. H. Wiebe and the author. The fungus was fruiting at the base of a large white oak in the Duke University Forest, Orange County, North Carolina, and when collected on July 20, 1952, the fructification was found to weigh 45 pounds. It was roughly circular in outline and measured from 23 to 26 inches (58 to 66 cm.) in diameter, with a height of 13 inches (33 cm.). The accompanying photograph shows the many imbricated pilei making up the fruiting body.

Other large fructifications of this species reported in the literature include the one Lloyd (Mycol. Notes, Polyporoid Issue, **3**: 36. 1910) received from Trenton, New Jersey, which weighed 43 pounds and measured 26.5 by 36 inches (67 by 91 cm.), with a height of 13 inches (33 cm.), and the one McIlvaine (One Thousand American Fungi) saw in Boston, which was 4 feet (1.2 m.) tall and 2.5 to 3 feet (76 to 91 cm.) wide. Since this species is commonly pictured with a very long underground stem, there is no way of knowing the relative proportion of stem to fruiting surface of the Boston specimen, and the weight was not given.—W. H. WILLS, Duke University.

NUTRITION OF MORCHELLA

In a paper published by the author (Mycologia **43**: 402–422. 1952) on the nutrition of *Morchella esculenta,* it was stated that little work had been done on the physiology of *Morchella.* Since that time it has been discovered that a paper published by Hans Hurni (The physiology of *Morchella esculenta.* Its synthesis of aneurine under various conditions. Mitt. naturforsch. Ges. Bern (N.S.) **4**: 11–18. 1947. (See also Chem. Absts. **45**: 9140) has covered many of the points that the present writer's paper investigated. Where similar things have been done, the results agree very closely.

It is interesting that two strains of *M. esculenta,* isolated from different parts of the world, should give the same nutritional responses. In a non-sporulating, non-fruiting mycelial form like *Morchella,* there is a paucity of morphological characteristics suitable for taxonomy, and it may be that nutritional responses may serve as an adequate adjunct to the usual methods.—THOMAS D. BROCK, Research Laboratories, The Upjohn Company.

THE NEW RULES

The International Code of Botanical Nomenclature (the title of what amounts to the fourth edition of the Rules) arrived early in November. Copies can be secured by those not members of the International Association for Plant Taxonomy for $3.70 (paper)

or $4.20 (buckram) through Dr. Reed C. Rollins, Gray Herbarium, 79 Garden Street, Cambridge 38, Massachusetts.

A NEW JAPANESE MYCOLOGICAL JOURNAL

Volume 1, No. 1 of NAGAOA, mycological journal of the Nagao Institute, 6–387, Kitashinagawa, Tokyo, Japan, has recently appeared. A preface and introductory outline indicate that the Institute is a private foundation for research in industrial mycology. NAGAOA is announced as biannual; the first number includes two papers of a series of "Enumerations of the Moulds of Japan," a first report of "Studies of the Sporobolomycetaceae in Japan" (all in English), an article "On the Genus *Graphiola* found in Japan" (Japanese with Latin diagnosis), and a forty-page "List of Cultures." The subscription price is $1.00 a year.

REVIEWS

THE YEASTS. A TAXONOMIC STUDY, by J. Lodder and J. J. W. Kreger-van Rij. xi + 713 pp. 268 figs. North Holland Publishing Co., Amsterdam; Interscience Publishers, Inc., New York. 1952. Price $19.50.

This remarkable compendium of all the yeasts, in one volume, provides an invaluable, authoritative guide and research tool for all who have any occasion to consider the taxonomy or nomenclature of these fungi. Although based upon the three volumes in which the authors have monographed the yeasts over a 20-year period, the book is entirely new. Modifications and additions over previous taxonomic works have been based upon and largely necessitated by modern advances, especially in our knowledge of the biology, genetics, and physiology of yeasts. The authors have been eminently qualified in this tremendous task not only in their own research experience but in the facilities at their command. At the Koninklÿke Nederlandsche Gisten Spiritusfabriek, and the Yeast Division of the Centraalbureau voor Schimmelculturs, both at Delft, there has been assembled one of the most extensive collections of yeasts available anywhere in the world.

The term yeast is used by the authors in its broad sense to include not only the ascosporogenous family, the Endomycetaceae, and the imperfect family, the Cryptococcaceae, but also the yeast-like fungi characterized by ballistospores and classified usually in the Sporobolomycetaceae. Some may question the propriety of including the Sporobolomycetaceae in a treatment of the yeasts. Certainly, however, its inclusion increases the utility of the book as a guide in determining the identity of any yeast-like organism.

In addition to its seven chapters, a section in the back of the book is devoted to Latin diagnoses of 15 new species, 4 new varieties, and 1 new genus. An author and a subject index are both provided as well as a list of references at the end of the chapters. The subjects taken up in each chapter are as follows: I. Intro-

duction. II. Characteristics and methods used in the classification. III. Variation in yeasts and its significance in taxonomy. IV. General classification of the yeasts. V. Discussion of the genera belonging to the Endomycetaceae. VI. Discussion of the genera Sporobolomyces and Bullera. VII. Discussion of the genera belonging to the Cryptococcaceae.

The initial division of the yeasts into three families is a morphological one, based on the formation of ascospores, ballistospores, or the lack of either, Genera, as well as the families, are also based essentially on morphological characters pertaining to vegetative and sexual reproduction. Species and subspecies groups, however, are established for the most part on physiological properties. This is in general accord with the views of other systematists working with yeasts and derives from a conviction that in practice it is impossible to base a system of yeast taxonomy on morphological characters alone. It is not a view, however, that will be accepted wholly by those mycologists who adhere to the morphological concept of species championed repeatedly by the International Congresses. On this basis, the yeast species are in effect physiologic races and the genera, morphologic species.

Foremost among the physiological tests used by the authors in separating species is the ability to ferment sugars with the formation of ethanol and carbon dioxide, employing such sugars as glucose, mannose, fructose, galactose, saccharose, maltose, lactose, raffinose and inulin. For the non-fermenting yeasts the property of using sugars as an assimilation and a respiration substrate has been the criterion in separating species. Other physiological properties, to be sure, have also been made use of, such as the assimilation of nitrogen, other carbon and sulfur compounds, amino acids and the vitamins.

One of the interesting features of the book is the analysis made of the different morphological and physiological properties of yeasts from the standpoint of their suitability as taxonomic criteria. The result has been the establishment of clearly set forth methods, carefully systematized and described, even to the details of the formulation and the preparation of media. In general, the yeasts were maintained on malt agar, and incubated at about 25° C. Purity was generally ascertained by the selection of single colonies from

dilution plates. Single cell transfers were resorted to in special cases.

Variation in yeasts was found by analysis to be largely of 3 types: changes in the genotype, cytoplasmic changes, and segregations. The genotype changes were considered to be hereditary and to have arisen by mutation in contrast to cytoplasmic changes which arise in the plasma or structural elements other than the nucleus. The changes from rough to smooth, likened to the S-R phenomenon in bacteria, accompanied by degeneration and loss of sporulation, are attributed to mutation but are reported to be reversible in some cases, stable in others. Those who have worked with mutations in other fungi may well question whether the mutations in yeasts are truly reversible when analyzed by the single cell culture method or, indeed, whether the changes referred to as reversible mutations are in fact true mutations.

Although remarkable changes in morphology or colony characters were attributed to mutation, these changes were generally accompanied by relatively slight changes in physiological characters, i.e., fermentative powers, the changes being mostly of a quantitative, seldom of a qualitative nature. The conclusion that physiological characters in the yeasts, more especially the fermentative properties, are fairly stable in spite of mutations strongly supports the use of such properties in the delimitation of their species or, for what mycologists in general would term, their races. Finally, after a discussion of the changes due to segregations, the authors find themselves neither in accord with Winge's "strictly orthodox Mendelian interpretation" nor with Lindegren's "rather revolutionary and confusing theory" in explanation of the various observations which have been made of segregation changes.

Apparently the facts of variation are found "rather depressive" for the yeast systematist. The problem is how to take into account in taxonomy the changes of one kind or another which are continually recurring and which, it is understandingly claimed, are not guaranteed against by starting with a single yeast cell. The cause of this dilemma, as seen by the authors, is that the morphological characteristics valued so highly in botanical taxonomy are very susceptible to all three types of variation. But is it not the real point that most botanists would not attempt to differentiate races

on morphological grounds? Yeast species are the horticultural varieties of the botanist.

With this introduction of the problems in yeast taxonomy and an explanation of the current species concept in yeasts, the authors launch into the actual systemics of yeasts in Chapter IV, with simply constructed keys for the separation of families and genera. Some 263 pages are devoted to the Endomycetaceae which includes keys, descriptions, diagnoses, historical surveys, synonyms, taxonomic discussions, often line drawings, and summaries and literature lists. The Sporobolomycetaceae take up 28 pages and the Cryptococcaceae 296 pages.

A total of 25 genera, 165 species and 18 varieties are recognized in this book, if we have added correctly. It is a refreshing experience to find the emphasis in a taxonomic treatment of fungi placed on synonymy rather than on the creation of new species.

Written in English of great clarity, simplicity, and directness, the book is designed in such a form as to be readily used by any biologist interested in yeasts. It is indispensable to the zymologist and yeast technologist. Every mycologist would want it as a reference book. What better tribute could a mycologist pay to this excellent work than to hope some day that other groups of the fungi may be treated with similar lucidity and precision.—WILLIAM C. SNYDER.

DISEASE IN PLANTS. An Introduction to Agricultural Phytopathology, by Neil E. Stevens and Russell B. Stevens. xx + 219 pp., 61 figs. plus additional cuts. Waltham, Mass., The Chronica Botanica Co., New York City: Stechert-Hafner, Inc. 1952. Price $4.75. Volume one of Chronica Botanica's "Manual of the Plant Sciences."

This is a unique and provocative book, well organized, very readable, clearly printed, and unusually free from mechanical errors. The book differs materially from other texts in plant pathology as details are omitted on specific diseases and techniques. Instead, the authors discuss plant pathology on the basis of broad, general principles. The book awakens the readers to the intimate relationship between plant pathology and related biological fields, but sometimes the related subject could be more effectively inte-

grated. Because of the general scope of the book, some may question its value as an elementary text, unless it is used in conjunction with a standard text in plant pathology.

The volume consists of twenty chapters covering a wide range of topics. Except for the first two chapters, they are grouped under three main headings: causal agents of plant diseases, factors influencing the development of pathogenic diseases, and disease control. In most cases only a small number of references are cited at the end of each chapter. Hence, the value of the book, particularly as a reference, could be materially increased, provided more original work were cited. The 61 illustrations, many of which have been published before, are in general of high quality; the volume also includes several cuts of historical interest. Although the book is intended primarily as an elementary text, it has even greater value for general professional and cultural reading.—J. J. CHRISTENSEN.

INTRODUCTORY MYCOLOGY, by C. J. Alexopoulos. 482 pp., 187 figs. John Wiley & Sons. 1952. Price, $7.00.

The author of this text-book of general mycology has certainly achieved his primary aim, which is to present an adequate treatment of the subject for a one-term course in mycology. For an introductory text, the book contains an unusual quantity of important details. With the addition of supplementary reading assignments, such as the references listed at the end of each chapter, the book could also be adapted for use in a two-term course. One of the most outstanding features of the book is the large number of illustrations, particularly the numerous life cycles illustrated with drawings selected from various research papers. It is noteworthy that the student is made aware of the more outstanding recent trends in the field of mycology. The writing is clear and not too technical, and the derivations of most of the technical terms are included. Also there is a very useful glossary of mycological terms.

The system of classification followed by Dr. Alexopoulos is fairly conventional and demonstrates his cognizance of recent trends in taxonomic research. It is commendable that the author has seen fit to include a chapter on the bacteria and actinomycetes, particu-

larly in view of the fact that it is becoming increasingly difficult to draw a sharp line of demarcation between these organisms and the fungi. Also included are the slime molds. The Fungi Imperfecti, an important group of organisms frequently neglected in mycological texts, are given adequate treatment. The Phycomycetes are placed in three main series—Uniflagellatae, Biflagellatae, and Aplanatae—with emphasis upon flagella, as advocated by Sparrow and by Karling. The Ascomycetes are treated in two sub-classes: the Protoascomycetes, including the Endomycetales and Taphrinales, and the Euascomycetes, including the Aspergillales, Myriangiales, Erysiphales, Laboulbeniales, Sphaeriales, Hypocreales, Microthyriales, Dothidiales, Pseudosphaeriales, Hysteriales, Ostropales, Helotiales, Pezizales, and Tuberales. The Erysiphales are placed in the Pyrenomycetes, rather than in the Plectomycetes, because of the regular arrangement of asci in the cleistothecium. The Mycosphaerellaceae and Pleosporaceae are included in the Pseudophaeriales, and the Phyllachoraceae are discussed under the Sphaeriales. The Basidiomycetes are divided into the two subclasses: Hemibasidiomycetes, including the Dacrymycetales, Tremellales, Auriculariales, Uredinales, and Ustilaginales, and the Holobasidiomycetes, which are divided into the conventional two series, Hymenomycetes and Gasteromycetes. All seven families of Hymenomycetes are placed in the single order, Agaricales, while the Gasteromycetes are divided into five orders. Throughout the text there are keys to the major groups (not including genera) of fungi.

There are some mistakes, most of which are not of a very serious nature. On more than one occasion it is stated that meiosis occurs in the first division of the zygote nucleus; whereas, two divisions are required for the completion of this process. If all of Blakeslee's investigations on sexual reproduction in the mucors are taken into consideration, it is very unlikely that one can conclude that Blakeslee regarded heterothallism as representing a true segregation of "sexes" in different thalli. Furthermore, it appears that most writers of mycological texts are unaware of a paper by Köhler, published in 1935, and one by Sjöwall, which appeared in 1946, that offer an explanation for the occurrence of sporangiospores of only one compatibility type in a single zygosporangium

of certain heterothallic mucors. Also, the coenzygotes of *Rhizopus nigricans* have now been germinated (by Sjöwall) and the life cycle has been demonstrated to resemble that of *Mucor mucedo*. In the discussion of the yeasts, it is unfortunate that the old concept of Wager and Peniston regarding the structure of the yeast nucleus is perpetuated, in view of the overwhelming recent evidence against it. It is erroneously stated that the heterokaryotic condition in *Neurospora sitophila,* with respect to mating types, may arise normally through hyphal fusions, and there are some discrepancies in the description of aeciospore formation in the rusts.

However, the mention of these errors should not be allowed to detract from the overall value of the book. It is a text which has long been needed for the teaching of a one-term course in mycology, and it has many important features which make it useful to more advanced students as well.—L. S. Olive.

Laboratory Manual for Introductory Mycology, by C. J. Alexopoulos and E. S. Beneke. 177 pp., 95 figs. Burgess Publishing Co., Minneapolis. 1952. Price, $3.25.

While primarily intended to be used in connection with the Alexopoulos or the Bessey text, this is suitable for general use. A brief introduction discusses preparation of temporary and permanent mounts, collection and preservation of fungi and culture methods. This is followed by a concise outline of the classification adopted, the principal divisions recognized being the bacteria, the slime molds and the true fungi. The exercises are not numbered and since the genera mentioned are for the most part those which are common and available in temperate regions generally, the instructor may use discreet selection of material to be studied. Each section is followed by a brief glossary and a selected list of special references. Few students will study all the genera mentioned but any who study a fair sample cannot fail to acquire a basic knowledge of the significant features of the major groups of fungi and an impression of the wide variability of the organisms included in that category.—G. W. M.

MYCOLOGIA

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CANDICIDIN, A NEW ANTIFUNGAL ANTIBIOTIC[1]

HUBERT LECHEVALIER, ROBERT F. ACKER, CHARLES T. CORKE, CONRAD M. HAENSELER AND SELMAN A. WAKSMAN

(WITH 4 FIGURES)

The rapid development of our knowledge of chemotherapeutic agents of microbial origin, known as antibiotics, has yielded in recent years a number of substances which have made possible the control of most bacterial and rickettsial infections. Relatively little progress has been made, however, in the control of virus and fungal diseases. These still remain a challenge to investigators in the field of antibiotics. The need for effective antifungal agents has been felt more strongly lately since a certain number of patients receiving antibiotics by the oral route of administration, for the control of bacterial infections, became more susceptible to secondary infections by fungi, notably yeast-like organisms (6). The potential importance of antibiotics active against phytopathogenic fungi may also be mentioned, even though such antibiotics have so far played only an insignificant role in the control of plant diseases (1).

ISOLATION OF CANDICIDIN-PRODUCING ORGANISM

A group of 197 cultures of actinomycetes belonging to the genus *Streptomyces*, isolated from soils and manures, were tested for their

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University—the State University of New Jersey, Departments of Microbiology and Plant Pathology.

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activity against *Ceratostomella ulmi*, the causative agent of Dutch elm disease. The cultures were streaked on plates containing potato or peptone-glucose agar media, and incubated for 2 to 4 days at 28° C. The plates were then cross-streaked with a fast-growing culture of *C. ulmi* (Pomerleau's strain), and further incubated at the same temperature for 4 to 5 days. The zone of inhibition of growth of *C. ulmi* was measured. The initial tests indicated that nearly 50 per cent of the isolates were antagonistic, in varying degrees, against the test organism.

Seven cultures, selected on the basis of their high consistent activity by the cross-streak method, were used for further study in liquid media. They were grown, in shake flasks, using nutrient and yeast glucose broths; in these media, four of the cultures showed no activity against bacteria (*Escherichia coli*, *Staphylococcus aureus*) or fungi (*C. ulmi*, *Aspergillus niger*). Two cultures exhibited both antibacterial and antifungal activity. Their antimicrobial spectra strongly indicated, however, that they were neomycin- and fradicin-producing strains, although culturally they were different from *Streptomyces fradiae* (3). The last of the seven active isolates yielded broths which possessed strong antifungal activity; this culture was, therefore, selected for further study. It was found to produce a new antibiotic substance which had no antibacterial activity but was characterized by marked fungistatic and fungicidal properties, especially against *Candida albicans*. It was designated, therefore, as ***candicidin***.[1a]

CHARACTERIZATION OF CANDICIDIN-PRODUCING ORGANISM

The candicidin-producing culture was isolated from a sample of cow manure during the summer of 1948. It belongs to the *Streptomyces griseus* group, although it showed certain minor differences from the typical streptomycin-producing strains of this organism (5). It has been deposited in the culture collection of the Department of Microbiology of Rutgers University as No. *3570*.

A study was made of the comparative cultural and morphological properties of a typical *S. griseus* and of No. *3570* culture. The

[1a] Preliminary data concerning this new antibiotic were presented at the Annual Meeting of The Mycological Society of America. Ithaca, September 10, 1952.

TABLE I

MORPHOLOGICAL AND CULTURAL PROPERTIES OF *S. griseus* AND NO. *3570*

	S. griseus	No. *3570*
Structure of aerial mycelium on yeast-dextrose and Czapek's agars	Branching filaments forming tufts of straight sporulating hyphae.	Branching filaments forming tufts of curved sporulating hyphae.
Gelatin stab	Rapid liquefaction. Greenish-yellow or cream colored surface growth with brownish tinge.	Rapid liquefaction. Flaky growth falls to bottom. Faint brownish pigment.
Czapek's agar	Thin, colorless, spreading, becoming olive buff. Aerial mycelium thick powdery, water green.	Poor growth, thin, cream colored, with brownish dark tinge in vegetative growth and greenish tinge in aerial mycelium.
Yeast dextrose agar	Cream colored growth elevated in center, gray-green powdery aerial mycelium.	Cream colored lichnoid growth, grey powdery aerial mycelium.
Nutrient agar	Abundant cream colored, lichnoid growth powdery, white to light grey aerial mycelium.	Cream colored growth thin, powdery, light grey aerial mycelium.
Glucose-asparagine	Thin cream colored growth. Light grey aerial mycelium. No soluble pigment.	Same.
Litmus milk	Cream colored ring. Coagulation with rapid peptonization, becoming alkaline.	Coagulation rapid with peptonization, becoming alkaline.
Potato plug	Brownish, lichnoid growth, covered with white powdery aerial mycelium. No soluble pigment.	Same.
Pigment	Not soluble in medium	Same.
Nitrate reduction	+	+
Optimum temperature	37° C	37° C
Sensitivity to phage M-1	Sensitive	Resistant
Sensitivity to streptomycin	100 mcg/ml	2 mcg/ml
Source	Garden soil	Cow manure

results are summarized in TABLE I. Culture *3570* forms tufts of sporulating hyphae characteristic of *S. griseus;* they differ from the typical forms only in that the aerial hyphae are slightly curved instead of being straight. Minor differences were also found in some of the cultural properties, such as a relatively poor growth of No. *3570* on Czapek's agar. These differences from the type species are no greater than those already reported (5) for different strains of *S. griseus.*

ASSAY AND STANDARIZATION OF CANDICIDIN

Candicidin did not diffuse readily in agar media; hence dilution assays were employed rather than the common diffusion methods. For routine procedures, the streak dilution method (4) was used. When there was a possibility of bacterial contamination, neomycin, in a concentration of 8 mcg per ml of medium,[2] was added. The same medium in liquid form was used for turbidimetric assays.

One candicidin unit was defined as the minimum amount of antibiotic per one ml of peptone-glucose agar which completely inhibited the growth of *Candida albicans 204.* An 18-hour-old culture of *C. albicans* grown on yeast-glucose agar was used as the inoculum.

The age of the *C. albicans* culture used as inoculum in the agar streak assay was found to cause no appreciable variation. No change in assay results was observed when the glucose concentration in the test agar was varied from 0.25 to 2.5%. The pH and the sodium chloride concentration of the medium were found, however, to have a marked effect on the value of the results obtained. The greater the pH and the sodium chloride concentration, within certain limits, the higher were the assay values obtained.

PRODUCTION OF CANDICIDIN

Candicidin was produced by growing culture No. *3570* on yeast-glucose medium,[3] at 28° C, in shake cultures, using 2000 ml Erlenmeyer flasks. A primary inoculum was prepared by transferring

[2] The medium consisted of 1% glucose, 0.5% peptone, 0.5% NaCl, 0.3% meat extract, 1.5% agar, in tap water. The pH was adjusted to give 7.2 after sterilization.

[3] Bacto yeast extract 1%, cerelose (commercial glucose) 1%, and tap water; pH adjusted before sterilization to 7.5 with 20% NaOH.

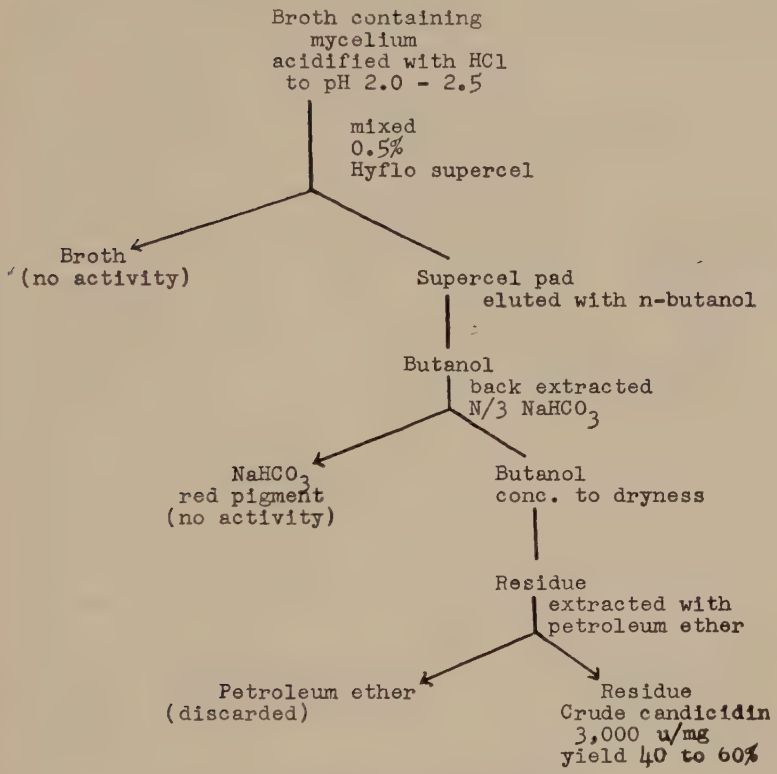

FIG. 1. Extraction of crude candicidin.

spores from agar slants into 250 ml Erlenmeyer flasks containing 100 ml medium. These flasks were shaken for 24 hours and the contents used to inoculate a series of similar flasks (secondary inoculum), which were then incubated for another 24 hours. The large Erlenmeyers, containing 300 ml of medium, were each inoculated with one of the cultures of the secondary inoculum and shaken at 28° C for 4 days. They yielded broths having candicidin potencies of 1000 to 5000 units per ml. Broths having potencies of 3000 to 5000 units were also obtained on a glutamic acid-glucose medium [4] in 250 ml flasks containing 100 ml of medium inoculated

[4] K_2HPO_4—0.5 gm, $MgSO_4 \cdot 7H_2O$—0.2 gm, $Fe(SO_4)_3 \cdot 9H_2O$—0.1 gm, $ZnSO_4 \cdot 7H_2O$—0.01 gm, anhydrous glucose—10 gms, L-glutamic acid—10 gms, distilled water 1000 ml; pH adjusted to 7.3 before autoclaving at 15 lbs for 15 minutes.

with a washed one-day-old yeast-glucose shake culture and incubated on a shaking machine, at 28° C, for 5 days.

EXTRACTION AND PURIFICATION OF CANDICIDIN

As a result of growth of the organism under the conditions outlined above, the culture attained a pH value of 7.5 to 8.5. The first candicidin preparations were made by extraction of the broth with n-butanol at pH 8.0. The butanol was concentrated to dryness and the residual solid suspended in water and freeze dried. The solid preparations obtained by this method had a potency of 900 units per mg.

A method of extraction giving materials having approximately three times this activity was later developed. The whole culture,

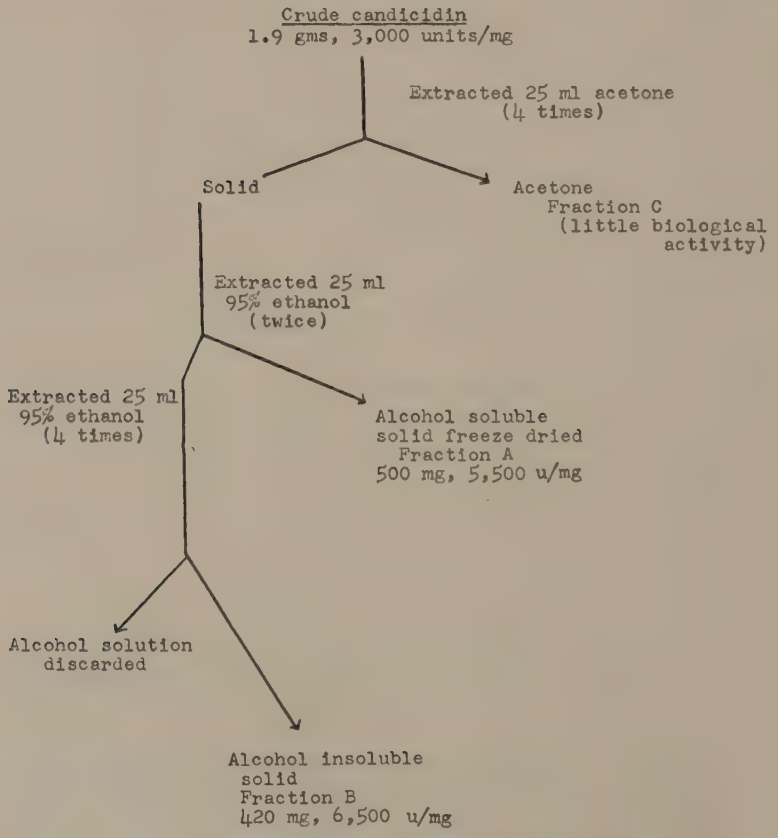

FIG. 2. Further purification of crude candicidin.

TABLE II

ANTIFUNGAL SPECTRA OF THREE FRACTIONS OBTAINED FROM CRUDE CANDICIDIN

Assay on peptone glucose medium. Cultures incubated, at 28° C, for 48 hours

	Dilution units per mg.		
	Fractions		
	A	B	C
Candida albicans	2,000	3,000	200
Ceratostomella ulmi	>10,000	>10,000	250
Trichoderma sp.	90	250	<30
Penicillium notatum	<90	<250	<30
Aspergillus niger	200	200	<30
Rhizopus nigricans	<90	<250	<30

including the mycelium, was treated with HCl to give a pH of 2.5, and stirred 10 minutes with 0.5 per cent Hyflo-supercel. The supercel pad, which was filtered off, retained the antibiotic present both in the broth and in the mycelium. An elution was made by mixing equal volumes of supercel and n-butanol. The solvent, which was deep red and contained the active substance, was filtered off. The pigment was removed by extracting the butanol exhaustively with 2 per cent of its volume of N/3 $NaHCO_3$. The residual butanol was concentrated to dryness *in vacuo* and extracted with petroleum ether. The active substance was suspended in water and freeze-dried (FIG. 1). The preparation obtained by this method was designated as "crude candicidin."

On further purification by the use of organic solvents, the crude candicidin yielded three fractions (FIG. 2). Two of these (A and B) had almost equivalent biological activity and could not be differentiated by their antimicrobial spectra (TABLE II); the third fraction (C) had negligible activity. The ultraviolet absorption spectra of the three fractions were found to be very similar, however (FIG. 3). When compared by paper chromatography,[5] fractions A and B gave Rf values varying from 0.4 to 8.0 (Fig. 4) and

[5] Method: ascending chromatography. System: 0.2% acetic acid in butanol saturated with water. Paper: Whatman No. 4. Development: 18 hours, at 23° C. Paper strips, after evaporation of solvent, plated out on assay medium seeded with an 18-hour-old culture of *C. albicans*. Sterile zones read after 18 hours of incubation at 37° C.

fraction C had an Rf value of 0.95. Fraction C diffused readily in the assay medium but had only a reducing effect on the growth of *C. albicans*, whereas fractions A and B did not diffuse from the paper but gave a sterile zone under the paper strip (FIG. 4).

PURIFICATION BY CHROMATOGRAPHY

Crude candicidin could also be purified by separation on a chromatographic column composed of cellulose powder. The column

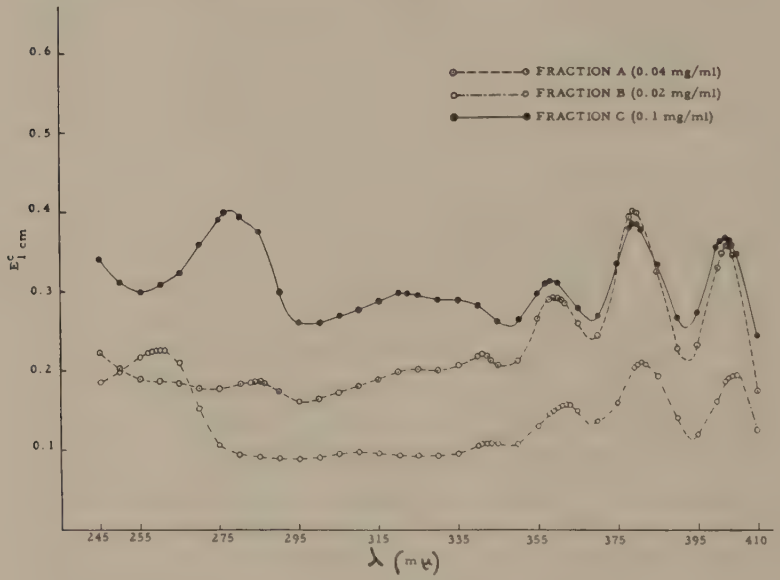

FIG. 3. Ultra-violet absorption spectra of candicidin fractions.

was prepared by pouring a water suspension of cellulose powder into a glass tube; while still moist, it was washed successively with equal amounts of ethylene glycol monomethyl ether, ethanol, and chloroform. The column was then loaded with a chloroform suspension of crude candicidin. The first elution was carried out with 95 per cent ethanol and yielded a reddish brown eluate, fraction C. Further elution was accomplished by the addition of an equal mixture of ethylene glycol monomethyl ether and ethanol. This mixture eluted first a reddish brown compound, fraction A, followed by a greenish compound, fraction B (TABLE III). So

far as could be determined from the comparison of their biological spectra, solubility properties, ultraviolet adsorption spectra, and Rf value upon paper chromatography, these three fractions A, B, and C, were identical with the corresponding fractions obtained by solvent extraction.

TABLE III

CHROMATOGRAPHY OF CRUDE CANDICIDIN ON CELLULOSE POWDER*

Column loaded with a chloroform suspension of crude candicidin (40,000,000 units), column size 2.5 × 70 cm

Solvent cut no.	Color	Solvent†	Volume ml	Assay units/ml	Fraction
1	yellow	chl	250	500	
2	yellow red	chl	10		
3	brown red	chl + EOH	25	4,000	
4	brown red	EOH	45		C
5	orange	EOH	30	2,500	
6	light orange	EOH	27		
7	yellow	EOH	95	500	
8	yellow	EOH	83	800	
9	brown red	EOH + EGMME	30	70,000	
10	brown	EOH + EGMME	90	70,000	A
11	green brown	EOH + EGMME	50	60,000	
12	green brown	EOH + EGMME	150	40,000	
13	green brown	EOH + EGMME	35	8,000	
14	green brown	EOH + EGMME	70	8,000	B
15	green yellow	EOH + EGMME	200	4,000	
16	green yellow	EOH + EGMME	200	1,000	
17	green yellow	EOH + EGMME	215	700	
18	green yellow	EOH + EGMME	300		

Total recovery (all factors) about 50%.

* Whatman ashless powder, chemically prepared, standard grade.
† Chl = chloroform
EOH = ethanol
EGMME = ethylene glycol monomethyl ether

ANTIMICROBIAL ACTIVITY OF CANDICIDIN

The antifungal activity of crude candicidin was assumed to be due to the antibiotic action of fractions A and B, since fraction C exhibited little or no activity. At the same time, the antibiotic spectra of A and B were found to differ only in degree of inhibition of the test organisms (TABLE II).

The spectrum of crude candicidin (TABLE IV) indicates no activity against bacteria, mycobacteria, or actinomycetes. This preparation was markedly active against yeasts and yeast-like fungi

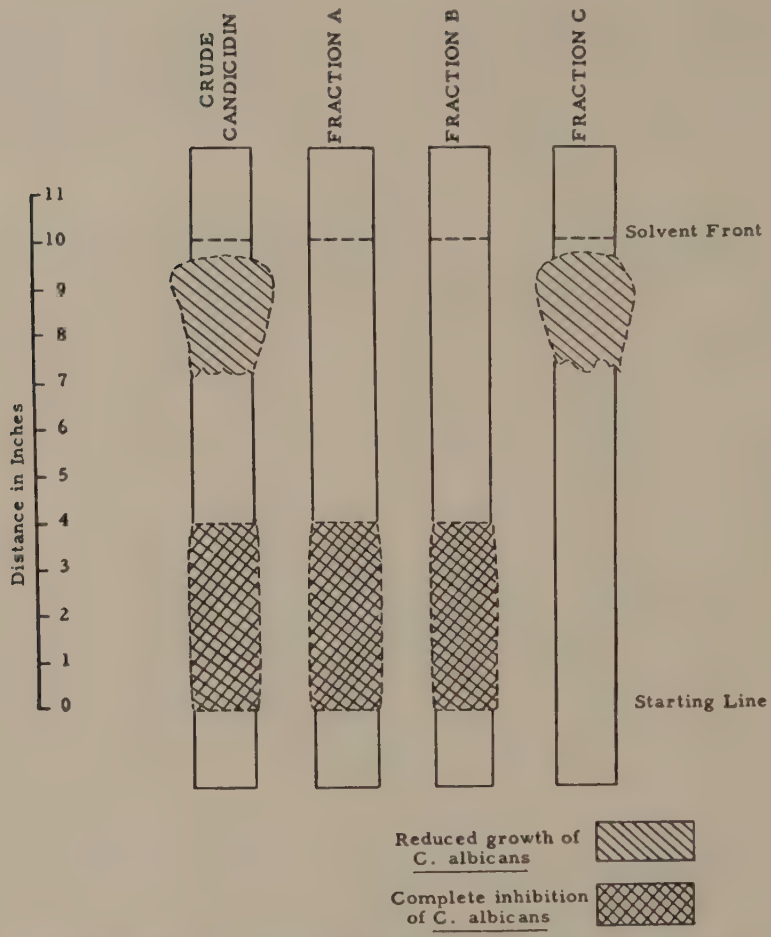

FIG. 4. Paper chromatography of candicidin fractions. Action of the developed paper strips on the growth of *C. albicans*.

(TABLE V). TABLE VI shows the antifungal activity of fraction B.

The fungicidal activity of candicidin was determined by mixing various amounts of crude material with a heavy suspension of *C. albicans* cells and allowing the mixture to stand at room temperature. Samples were taken at regular intervals and streaked on peptone-glucose agar to determine viability of the cells. Apparent sterilization of a suspension of resting cells was effected in 3 hours with a concentration of 5 mcg/ml; in the case of growing

TABLE IV

ANTIBACTERIAL SPECTRUM OF CRUDE CANDICIDIN

Streak dilution assay carried out on nutrient agar
Incubation at 37° C for 18–48 hours

Name of organism	Micrograms per ml for complete inhibition
Escherichia coli	>100
Aerobacter aerogenes	>85
Serratia marcescens	>85
Pseudomonas fluorescens	>85
Bodenheimer's organism	>85
Staphylococcus aureus	>100
Bacillus subtilis	>100
Mycobacterium phlei	>85
M. avium	>85
Mycobacterium sp. 607	>85
Streptomyces fradiae	>85

TABLE V

ANTIFUNGAL SPECTRUM OF CRUDE CANDICIDIN

Test on peptone-glucose medium. Incubation for 2 to 5 days at 28° C

	Microgram/per ml for complete inhibition
Acrostalagmus sp.	>10
Alternaria sp.	0.6–10
Aspergillus niger	5–70
Candida albicans	0.3–0.5
Cercospora kikuchii	8–15
Ceratostomella ulmi (P strain)	0.15–0.3
C. ulmi (H strain)	3–5
Botrytis sp.	16–50
Diaporthe sp.	13–70
Epicoccum sp.	30–50
Helminthosporium sp.	13–20
Isaria sp.	>100
Fusarium sp.	66–>100
Hormodendron sp.	30–50
Oospora sp.	>100
Mucor sp.	3–10
Penicillium notatum	5–100
Pestalozzia sp.	0.6
Phoma sp.	66–100
Polyporus sulphureus	8–20
Pullularia sp.	1–3
Pythium sp.	66–100
Saccharomyces cerevisiae	<0.15–0.3
Sclerotium rolfsii	>100
Spicaria sp.	>100
Stemphylium sp.	>85
Stysanus sp.	>100
Trichoderma sp.	20–30
Ustilago zeae	1–20
Verticillium sp.	0.6–>100

TABLE VI

ANTIFUNGAL SPECTRUM OF FRACTION B

Test run on peptone-glucose agar
* incubated at 28° C
† incubated at 37° C
Results in micrograms per ml for complete inhibition

Organism	Days of incubation 1	5
* *Aspergillus niger*	2	>10
† *Candida albicans*	0.22	1.1
† *C. brumptii*	3.3	>10
† *C. kruseii*	1	2.2
† *C. neoformans*		0.5
† *C. pseudotropicalis*	1.7	>10
† *C. stellatoides*	0.41	1.1
† *C. tropicalis*	2	>10
* *Ceratostomella ulmi* (P strain)		1.1
† *Cryptococcus neoformans*		0.5
* *Fusarium* sp.		10
* *Hormodendrum pedrosoi*		>10
* *Mucor* sp.	0.1	>10
* *Penicillium notatum*		1.1
* *Phialophora verrucosa*		>10
* *Rhizopus nigricans*	>10	>10
* *Saccharomyces cerevisiae*	0.06	0.14
* *Sporotrichum schenkii*		>10
* *Trichophyton gypsum*		>10
* *T. mentagrophytes*		>10

cells 1 mcg/ml was sufficient to sterilize the culture in 3 hours (TABLE VII). When *C. ulmi* and *A. niger* were tested in a similar manner, little fungicidal activity was observed.

TABLE VII

FUNGICIDAL EFFECT OF CANDICIDIN ON RESTING CELLS OF *Candida albicans*

Cell suspension of *C. albicans* in sterile water, treated with various concentrations of crude candicidin, for varying periods of time, transferred to peptone-glucose agar and incubated at 37° C for 18 hours to determine viability.

mcg/ml	Exposure to candicidin in hours 1	2	3	7	20
Control	++++	++++	++++	++	++
250	−	−	−	−	−
100	−	−	−	−	−
50	−	−	−	−	−
10	−	−	−	−	−
5	+	+	−	−	−
1	++	++	+	+	±
0.5	+++	+++	++	++	+
0.1	++++	+++	+++	++	+

PHYSICAL AND CHEMICAL PROPERTIES OF CANDICIDIN

Crude candicidin was soluble in the higher alcohols, but insoluble in benzene, petroleum ether, carbon tetrachloride, xylene, carbon disulfide, ethylene dichloride, ether, or ethyl acetate. Only fraction A was soluble in water (TABLE VIII).

TABLE VIII

SOLUBILITY OF 3 CANDICIDIN FRACTIONS

	Crude candicidin	Fraction		
		A	B	C
Water	+−	+	−	−
Ethanol	+−	+	−	+
Butanol	+	+	+*	+
Glycerol	+			
Benzyl alcohol	+			
Ethylene glycol (EG)	+	+	+*	+
EG monomethyl ether	+	+	+*	+
Chloroform	+−			
Acetone	+−	−	−	+

* Solubility of fraction B solid preparations in these solvents about 100 mcg/ml.

\+ = soluble
− = insoluble
+− = partially soluble

Concentrated solutions of candicidin were more stable than dilute solutions. At neutrality, fractions A and B withstood heating for 10 minutes at 60° C and could be kept for a week at 4° C without appreciable loss of activity. TABLE IX illustrates the stability of solutions of candicidin fractions A, B, and C at room temperature.

TABLE IX

STABILITY OF CANDICIDIN SOLUTIONS AT ROOM TEMPERATURE
Candicidin units per ml

Fraction	Hours at 27° C				
	0	3	9	24	168
A	6000	4500	2500	1000	900
B*	6000	4500	2000	1500	1500
C	250	250	200	100	200

Solutions in ethylene glycol monomethyl ether containing 1 mg/ml.
* B solution filtered off on paper.

The three fractions of candicidin had very similar U. V. absorption spectra (FIG. 3), which suggests a close chemical relationship between these fractions. The following peaks of maximum absorption were recorded:

Fraction A: 360, 380, 403 mμ
Fraction B: 362, 381, 404 mμ
Fraction C: 358, 379, 402 mμ

Elementary analysis of fraction A gave the following values on an ash-free basis: H = 9.6%, C = 62.9% and N = 4.7%. Fraction B was somewhat different: H = 9.9%, C = 57.8% and N = 7.3%. Some sulfur could also be detected in both fractions but it is very possible that it was present as an impurity in the ash.

Crude candicidin did not dialyze through a cellophane membrane.

TOXICITY OF CANDICIDIN TO EXPERIMENTAL ANIMALS

Acute toxicity testing of various candicidin preparations, using twenty gram albino mice, gave the following LD_{50} by the subcutaneous route of injection: crude candicidin 663 mg/kg, fraction A 277 mg/kg, fraction B 159 mg/kg. Intraperitoneally the following LD_{50} were obtained: crude candicidin 79 mg/kg, fraction A 47 mg/kg, fraction B 53 mg/kg. Necrosis was noted at the site of inoculation when the concentration of crude candicidin reached 0.63 mg per 20 gm mouse. Necrosis was also observed when 0.16 mg or more of fraction A or 0.32 mg or more of fraction B was injected per mouse.

EFFECT OF CANDICIDIN ON PLANT GROWTH

Crude candicidin was found to have no ill effect upon the germination of pea seeds in concentrations of 125 mcg/ml or less. Two ml of the candicidin solution were added to 5.5 cm petri dishes containing 5 to 10 pea seeds. These dishes were placed inside regular size (9.0 cm) dishes containing an absorbent pad saturated with water. The seeds were incubated 5 to 6 days at 20° C (TABLE X).

Spraying young bean plants once a week with an aqueous suspension of crude candicidin (660 mcg/ml) resulted in a decrease

TABLE X

EFFECT OF CRUDE CANDICIDIN ON GERMINATION OF PEA SEEDS

	Candicidin	Germination	Root growth, in per cent of control
	mcg/per plate	*per cent*	
No pretreatment	†1000	40	43
	†500	100	54
	250	100	120
	100	100	100
	50	100	103
Seeds soaked in water 24 hours prior to testing with candicidin	†1000*	100	90
	†500*	100	90
	250	100	103
	100	100	114
	50	100	103

* Development of primary leaves inhibited at these concentrations.
† With other batches of peas, no reduction of the root length and no inhibition of the primary leaves were observed at these concentrations.

of a powdery mildew infection. The spraying was started about 5 days before the mildew infection was apparent on the plant leaves. One single spraying before the appearance of the mildew did not show any therapeutic value. No toxic reactions were noted.

COMPARISON OF CANDICIDIN WITH OTHER ANTIFUNGAL ANTIBIOTICS PRODUCED BY ACTINOMYCETES

Candicidin was compared with other antibiotics produced by actinomycetes which were antifungal and lacked antibacterial activity. The ultraviolet absorption spectra of the candicidin fractions were distinctly different from the spectra for actidione, antimycin A, fradicin, fungicidin, and rimocidin. No direct comparison was made between candicidin and actinone, but a comparison of the data of Ikeda *et al.* (2) with our own showed that actinone was less toxic and less active *in vitro* than was candicidin. The solubility properties and the nitrogen content of the two substances were also different.

ACKNOWLEDGMENT

The authors are greatly indebted to Dr. W. Ruigh, formerly of the Department of Microbiology of Rutgers University, who devised part of the method of extraction of crude candicidin reported

in this paper and to Dr. V. Groupé and Miss L. Pugh, who determined the acute toxicity of the various preparations. The advice of Dr. Robert L. Peck and Dr. K. Folkers of Merck and Company was most valuable during this investigation. The authors also wish to thank Mr. Edwin Bailey and Mr. Aldrage B. Cooper for their technical assistance.

SUMMARY

A group of 197 cultures of actinomycetes were tested for their antibiotic activity against *Cerastostomella ulmi*. This screening program yielded a strain of *Streptomyces griseus* which produced an antibiotic substance that was very active against yeasts, yeast-like fungi, and *C. ulmi;* it was not very active against filamentous fungi and had no activity against the bacteria tested. The new antibiotic was different from the known antifungal agents actidione, antimycin A, fradicin, fungicidin, rimocidin and actinone.

Because of its marked fungistatic and fungicidal action against *Candida albicans*, the new antibiotic was designated as candicidin. A crude extract of candicidin could be obtained from liquid culture media, by adsorption on Hyflo-supercel and elution with n-butanol. Crude candicidin could be further fractionated, by solvent extraction and chromatography on cellulose powder, into two very active fractions (A, B) and an almost inactive fraction C.

Crude candicidin was fungicidal against growing and resting cells of *Candida albicans* in the concentration of 1 to 5 mcg/ml. It had no injurious effect upon the germination of pea seeds in concentration of 125 mcg/ml. Spraying young bean plants once a week with an aqueous suspension of crude candicidin (660 mcg/ml) resulted in a decrease of a mildew infection.

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PIGMENT PRODUCTION IN CERTAIN OF THE ASPERGILLUS GLAUCUS GROUP [1, 2]

GEORGE THOMAS JOHNSON [3] AND BERNARD S. GOULD

(WITH 8 FIGURES)

Gould and Raistrick (1934) have shown that the *Aspergillus glaucus* group, perhaps the most common of the Aspergilli, are characterized by the production of several crystalline pigments: flavoglaucin and auroglaucin (whose structures are given in FIG. 1), as well as a series of substituted anthraquinones and anthranols (Raistrick, 1950). Flavoglaucin and auroglaucin are substituted

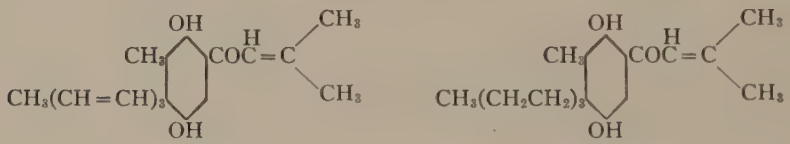

FIG. 1. Structural formulae of the characteristic pigments of the *A. glaucus* group.

toluquinol structures. The present study deals primarily with these latter pigments. Auroglaucin appears to be an oxidation product of flavoglaucin which poses the question as to whether or not these compounds constitute an oxidation-reduction mechanism in the organism. While anthraquinones and anthranols are produced in but relatively small quantity by these molds, flavoglaucin and auroglaucin together can be produced to the extent of 40% or more of the dry weight of the organism. The fact that these pigments perhaps constitute the principal end product of metabolism greatly increases interest in the substances. Flavoglaucin and auro-

[1] This investigation was supported in part by a grant from the A. C. Lawrence Leather Company of Peabody, Massachusetts.

[2] A preliminary report was presented at the Gordon Conference on Microbiological Deterioration, July 2–6, 1951.

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glaucin have been isolated only from members of the *Aspergillus glaucus* group, but compounds of closely related structure have been obtained from other organisms in relatively small yields.[4]

The fact that these pigments constitute so significant a part of the end products of metabolism suggested that a study of the physiology of pigment production might throw some light on the biosynthetic mechanisms involved in the formation of relatively complex molecules from simple carbon sources such as glucose. While Gould and Raistrick (**1934**) in their study of the pigments defined some of the conditions which lead to an increase in pigment production, no attempt to elucidate the mechanism of pigment formation by members of this group has yet been made.

In the *glaucus* group pigment production is extremely good on common carbohydrate sources; further, these Aspergilli are, in general, noted for producing small amounts of ethyl alcohol. Hence it was felt that the problem of pigment biosynthesis might be approached by determining whether disruption of the usually accepted pathways of carbohydrate dissimilation, such as those leading to ethyl alcohol in yeast metabolism, would lead to concomitant interruption of pigment production in species of *Aspergillus*. Results of experiments with the commonly used inhibitors (such as sodium fluoride and iodoacetic acid; cyanide and azide) are reported in the present paper. Studies are also reported on the availability of numerous substances as possible precursors of the pigments. In view of the results obtained during the course of this investigation, particular emphasis was directed toward the possible roles of mannitol, which is so ubiquitous a mold metabolic product, and of glycerol.

MATERIALS AND METHODS[5]

Organism. The organisms used in these studies were *Aspergillus novus* (NRRL Culture No. *46*) and *Aspergillus pseudoglaucus* (NRRL Culture No. *41*), both obtained from Dr. Kenneth

[4] Spinulosin from *Penicillium spinulosum*, *Aspergillus fumigatus*, and *P. cinerascens* (Birkinshaw and Raistrick, **1931**; Anslow and Raistrick, **1938**), fumigatin and 3-hydroxy-4-methoxy toluquinol from *A. fumigatus* (Anslow and Raistrick, **1938**).

[5] We are indebted to Mrs. Ruth Michaels for technical assistance with certain phases of this investigation.

B. Raper of the Northern Regional Research Laboratory, U. S. Department of Agriculture, Peoria, Illinois, to whom we express our thanks.[6] Most of the work to be presented was carried out with *A. pseudoglaucus* since it gave the most consistent growth and was most adaptable to submerged growth. Confirmatory experiments were frequently carried out with *A. novus*. Spore suspensions used for inoculation were made from growths developing on sporulation agar (formulation of Moyer and Coghill, **1946**). The spores produced on each test tube slant were suspended in 15 ml. of sterile water.

Medium. Most of the experiments were carried out in media having the basal salt mixture of Czapek-Dox medium: KH_2PO_4, 1.0 g.; KCl, 0.5 g.; $MgSO_4 \cdot 7H_2O$, 0.5 g.; $FeSO_4 \cdot 7H_2O$, 0.01 g.; $NaNO_3$, 2.0 g.; water, 1000 ml. The desired carbon source was added as indicated in each experiment reported. Gould and Raistrick (**1934**) found that ammonium salts of organic acids such as ammonium tartrate gave the highest yields of pigment under the growth conditions they had tested. In the present studies, however, such carbon-containing nitrogen sources were avoided since their use would complicate any interpretation of the results obtained. Usually 75 ml. quantities of medium were dispensed in 300 ml. Erlenmeyer flasks and sterilized in an autoclave for 20 minutes at 15 lbs. pressure.

Cultural Conditions. Surface growths. Flasks were inoculated with uniform spore suspensions from sporulation agar using 1 ml. of spore suspension. The flasks were incubated without agitation at $24° \pm 2°$ C. They were exposed to both light and dark.

Submerged cultures. The flasks were inoculated as described above, then placed on a shaking machine where they were continuously agitated. The growths so produced were made up of uniform pellets which remained colorless for about four days and then, over a period of a few days, assumed a uniform brilliant yellow color.

[6] Thom and Raper (**1945**) consider these cultures two strains of a single species (*A. pseudoglaucus*). However the two cultures often present striking biochemical peculiarities and we have distinguished them under the original strain names in the present paper.

Upon extraction the typical flavoglaucin and auroglaucin could be isolated and identified.

Resting preparations from submerged cultures. The submerged pellets constituted a convenient material for studying pigment production, particularly on substrates which did not support good growth. What will be called "resting preparations" were prepared in the following manner. After three or four days growth on the shaker and while the pellets were still colorless, the growths were combined and centrifuged to remove the original growth medium. The collected pellets were then resuspended in sterile solutions of the same composition as the original medium on which they had been grown and washed by centrifugation. This procedure was repeated until the pellets had been washed three times in fresh solutions. Washed "resting preparations" were then resuspended in various substrates, as desired, for determination of the availability of these substrates for growth and pigment production. Aseptic precautions were taken throughout.

Chemical Methods. Estimation of growth. At the end of the desired growth period the mycelium was filtered off, washed thoroughly with cold distilled water, pressed as dry as possible between paper towels, and dried to constant weight at 40° C. This was termed the dry weight.

Extraction of pigments. Total crude pigment. The dried mycelium was mixed with sand, ground to a fine powder in a mortar, and continuously extracted with anhydrous ether in a Soxhlet extractor until no further pigment was removed. The ether extract was evaporated to dryness and the residue was dried in a desiccator to constant weight. This is taken as total crude pigment. A certain amount of fat is included but examination of the extracts indicated that the small amount produced constituted a relatively small percentage of the total crude pigment in all cases.

Fractionation of flavoglaucin and auroglaucin. The ether-extracted crude pigment was refluxed with a small volume of hot pentane or light petroleum (boiling point 50–60° C.). The flavoglaucin can be crystallized from the petroleum extract and the residue which is largely auroglaucin is crystallized from methyl alcohol.

EXPERIMENTAL RESULTS

Effect of concentration of carbon source on growth and pigment production

The concentration of the carbon source markedly influences both growth and pigment production as indicated by the results obtained with surface cultures shown in FIG. 2. Within wide limits, increasing carbon concentration increases both the total growth as well as total pigment. The figure shows the effects of raising the concentration to 20%. Further increases to 50% sugar concentration re-

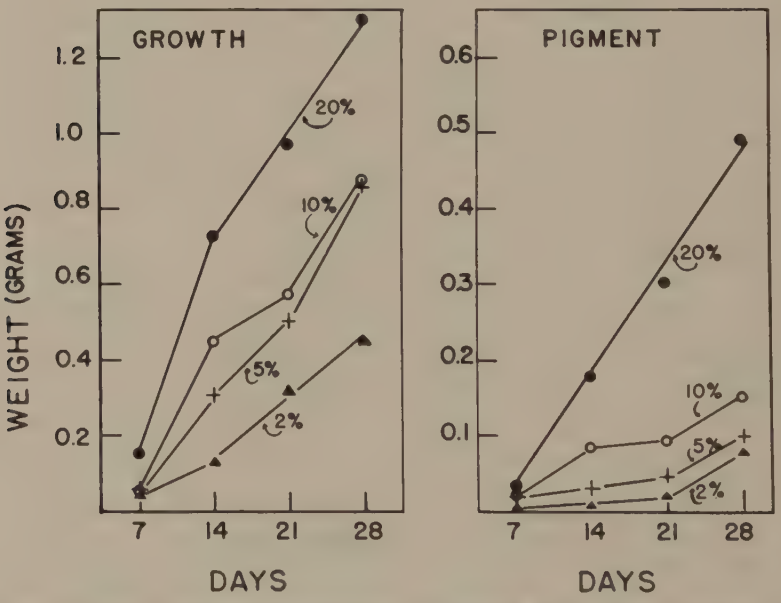

FIG. 2. Effect of glucose concentration on growth and pigment production. *A. pseudoglaucus*. Surface cultures.

sult in further increases of both growth and pigment and the optimum sugar concentration for these processes on glucose medium is above this point. The results shown in FIG. 3 for an analogous experiment with glycerol as the carbon source are strikingly similar.

Availability of various carbon sources for growth and pigment production on surface culture

The results of a typical experiment where surface growths developed on media containing 5% of the desired carbon source are illus-

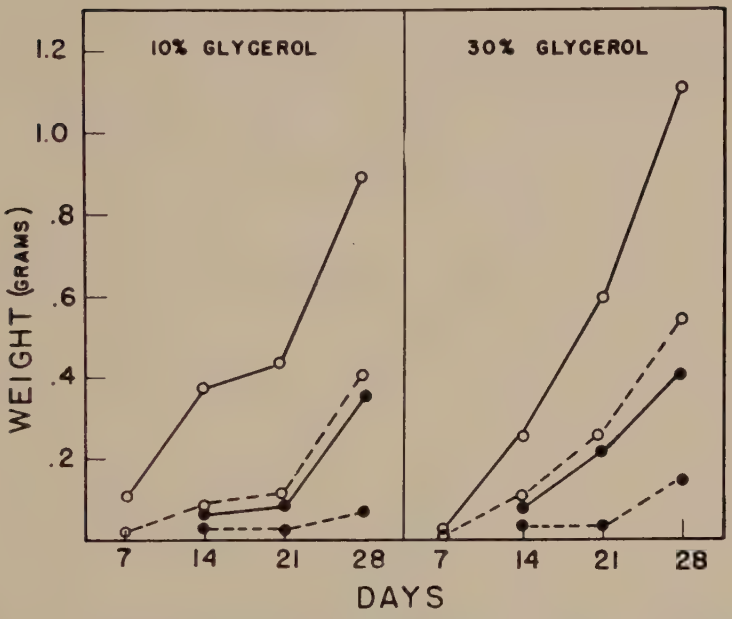

FIG. 3. Effect of glycerol concentration on growth and pigment production. *A. pseudoglaucus.* Surface cultures.
-○———○- = Growth; -○- - -○- = Total pigment;
-●———●- = Pentane; -●- - -●- = Ether fraction.

trated in TABLE I. After 21 days' incubation all of the carbon compounds studied yielded significant growth. Considerable variation in both total growth and pigment production occurred. As a result the amount of pigment per gram of dry mycelium was taken as the

TABLE I

GROWTH AND PIGMENT PRODUCTION BY ASPERGILLUS PSEUDOGLAUCUS ON VARIOUS CARBON SOURCES (SURFACE GROWTHS)

Carbon source*	Total growth g. per flask (dry weight)	Pigment per gram of mycelial growth (dry wt. basis)
Glucose	1.050	.187
Mannose	1.290	.265
Galactose	.913	.243
Fructose	.942	.123
Xylose	1.190	.346
Glycerol	.707	.389
Mannitol	.563	.320
Sorbitol	.781	.055

* Czapek-Dox medium with 5% of carbon source. Temperature 24° C. Grown for 21 days.

most revealing index of the relationship between a carbon source and pigment production. An examination of such values described in TABLE I indicates that glycerol, mannitol, and xylose yielded almost twice the amount of pigment per gram of mycelium as did glucose. Other experiments, one of which is presented in FIG. 4, also suggest a probable preferential role for compounds such as

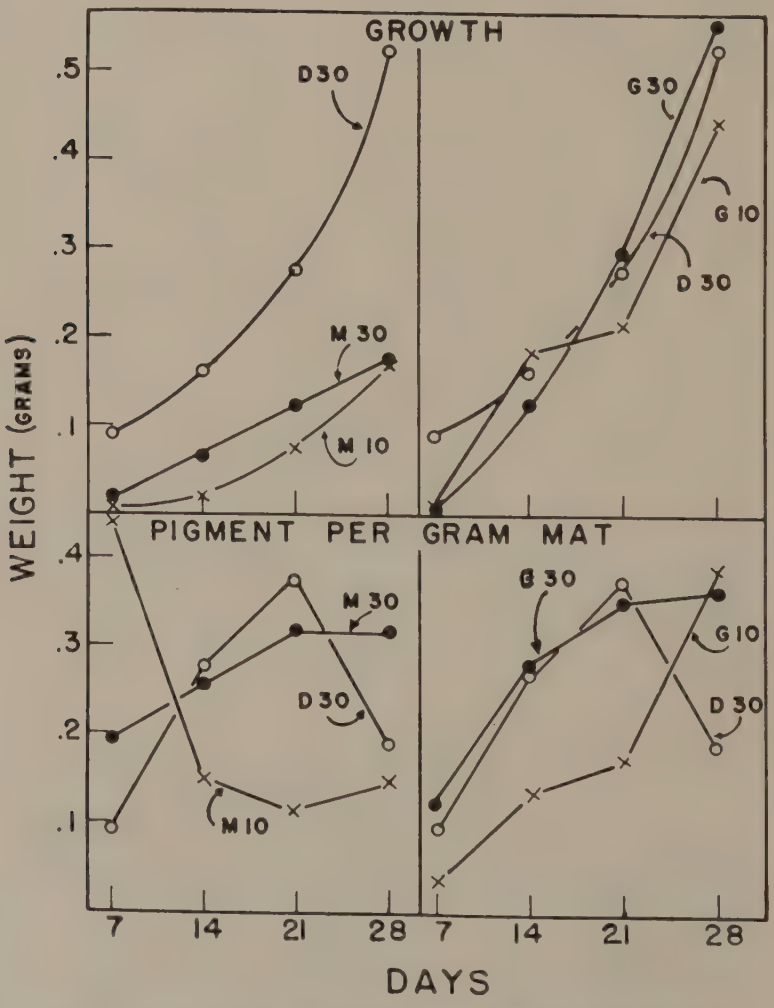

FIG. 4. Effect of carbon sources on growth and pigment production. *A. pseudoglaucus.* Surface cultures.
D = Glucose; M = Mannitol; G = Glycerol.

glycerol and mannitol in pigment synthesis. Despite the fact that growth on mannitol is markedly less, in this experiment production of pigment per gram of mycelium is as good in glycerol and mannitol as in the glucose growths.

Growth and pigment production in submerged culture

In order to avoid the difficulties usually encountered in metabolic experiments involving surface cultures of molds, experiments utilizing submerged cultures were carried out. Some comparisons of typical results which may be obtained with both surface and submerged cultures of *A. novus* and *A. pseudoglaucus* grown on glucose are shown in FIG. 5. In submerged culture the time required for an amount of growth equivalent to that obtained in surface cultures is greatly reduced; growth in submerged cultures at the end of 14 days will appreciably exceed surface growths in most cases. Pigment is produced in submerged cultures but the total pigment produced per culture and particularly the total pigment per gram of growth is markedly reduced in comparison with surface growths. In spite of the decrease in pigment it remains a significant amount. Also, in spite of the poorer pigment production, submerged conditions of cultivation were indicated for further studies because of the desirable characteristics of the growth. In shake flasks, for 3 to 4 days after inoculation, the growth consists of uniform, colorless pellets. Further, beginning about the fourth day, the pellets become colored, starting slowly and increasing in intensity over a four day period. This provides a semi-quantitative index of the rate of pigment production during the course of growth, yielding data which supplement the more quantitative data obtained upon extraction of pigment from the mycelium at the termination of the experiment. Since all pellets are more or less continuously submerged, each pellet on a statistical basis is subject to almost identical conditions of medium, etc. This is to be contrasted with the more variable conditions that obtain with surface cultures where some of the culture is submerged and some is aerial. The submerged growth makes it possible to subject the entire organism uniformly to the selected substrate or inhibitors (to be described below) in a way that is not possible with surface growths.

The results of some further experiments on growth and pigment production in submerged cultures are given in FIG. 6. Here pigment production on glucose and on glycerol are compared under the same environmental conditions. It is striking that, in this experiment, while the pigment per gram of organism is but 10% on glucose, it approaches 60% on glycerol. This suggests again that glyc-

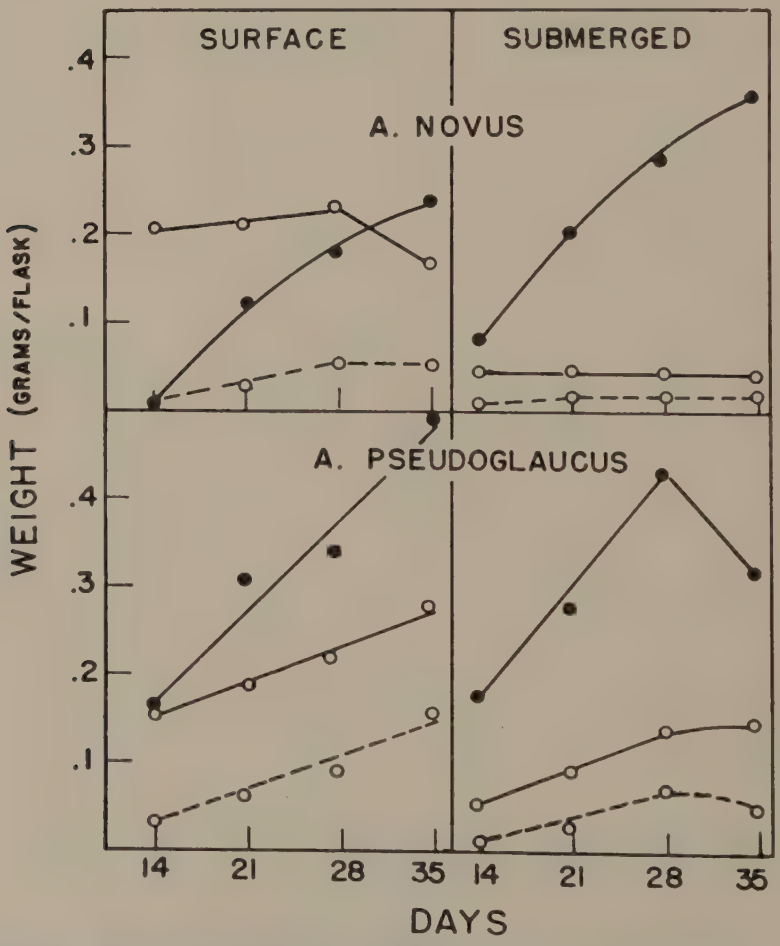

FIG. 5. Comparison of growth and pigment production in surface and submerged cultures. Czapek-Dox medium (10% glucose).
-●——●- = Growth; -○---○- = Pigment;
-○——○- = Pigment /gm. mat.

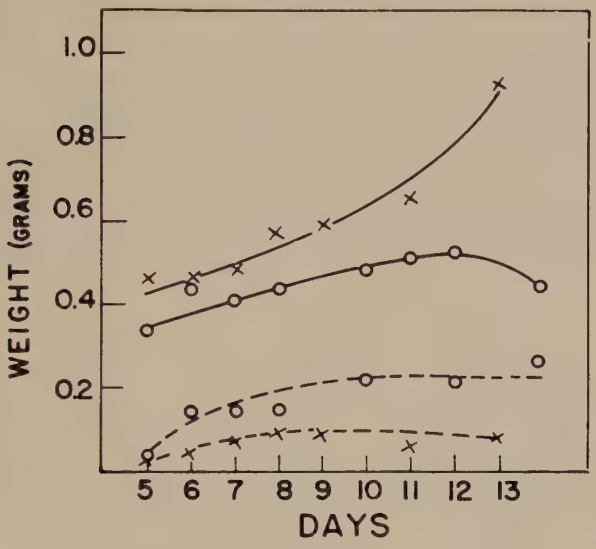

FIG. 6. Growth and pigment production in submerged cultures. *A. pseudoglaucus.* Czapek-Dox medium. × = 10% glucose; ○ = 10% glycerol; ——— = Growth; – – – – = Pigment.

erol or some closely related compound may be particularly significant in pigment synthesis.

Inhibition with fluoride and iodoacetate

The results obtained from a study of pigment production on glycerol suggest that when the organism is grown on glucose the latter may be dissimilated to three carbon residues by the usual glycolytic mechanisms and that these residues may then be built into the more complex pigment molecules. Experiments designed to disrupt the usual pathways for the breakdown of glucose to three carbon residues were set up of which the following is typical. Shake cultures of *A. pseudoglaucus* were allowed to develop for three days until good colorless pellets had been obtained. The inhibitors were added at this time in the concentrations indicated and the cultures were allowed to develop for a total of ten days. At this time growth and pigment production were determined. The results are given in FIG. 7. It is apparent that pigment production occurs on glycerol in the presence of inhibitor concentrations far greater than is

required to inhibit pigment production when glucose is the source of carbon. Both fluoride and iodoacetate stop growth and pigment production on glucose in concentrations that have no effect on the organism developing on glycerol. Experiments which confirm this data and more narrowly delimit the point of pigment inhibition are reported in TABLE II. It can be noted that M/5000 and M/10,000 iodoacetate usually inhibit both growth and pigment production on glucose while M/1000 iodoacetate did not inhibit either growth or

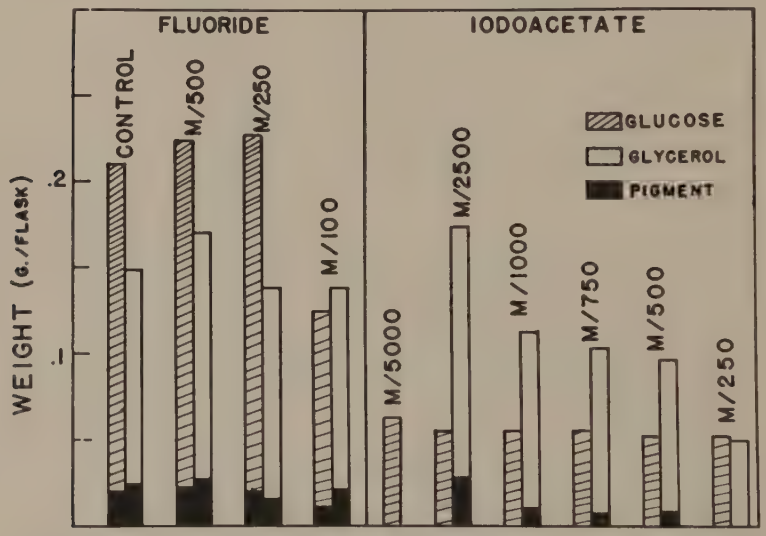

FIG. 7. Effect of sodium fluoride and iodoacetate on growth and pigment production. *A. pseudoglaucus*. Submerged growths. Czapek-Dox medium. Total incubation 10 days.

pigment production on glycerol. Similarly M/250 sodium fluoride inhibited pigment production on glucose while concentrations as high as M/25 fluoride failed to inhibit production on glycerol.

Inhibition of aerobic mechanisms by cyanide and azide and the role of oxygen in pigment production

From the data reported in TABLE II it became apparent that inhibition of the aerobic pathways of carbohydrate metabolism by sodium cyanide or sodium azide has a drastic effect both on glucose

and glycerol metabolism, suggesting that in all probability oxidative metabolism is essential for pigment production. The effects observed with sodium azide were consistently reproducible but those with cyanide were not. Some concentrations of cyanide resulted in good pigment production and there is a suggestion that under some conditions cyanide can be used as a carbon or nitrogen source by the species being studied. Similar results have been obtained in experiments with Fusaria (Gould and Tytell, **1941**).

TABLE II

EFFECT OF INHIBITORS ON GROWTH AND PIGMENT PRODUCTION IN SUBMERGED CULTURES

(*Aspergillus pseudoglaucus*. Data for Growth* on Glucose and Glycerol)

Inhibitor concentration	Sodium fluoride		Iodoacetate		Azide		Cyanide	
	Glucose	Glycerol	Glucose	Glycerol	Glucose	Glycerol	Glucose	Glycerol
M/25	−	+					−	−
M/50	−	+					−	−
M/100	±	+	−	−			−	−
M/250	+	+	−	−			±	−
M/500	+	+	−	−				
M/750			−	±	−	−		
M/1000			−	+	−	−		
M/2500			−	+	−	−		
M/5000			±	+	−	−		
M/10,000			±	+	−	−		
M/25,000			+	+	−	−		

* Medium Czapek-Dox with 10% of carbon source. Ten days' incubation.
\+ Definite pigment production.
− No pigment production.
± Trace of pigment.

To obtain additional data, therefore, the role of oxygen in pigment production was investigated in two additional ways. In the first of these the atmosphere above the organisms growing in submerged culture on the shaking apparatus was replaced by nitrogen gas before pigment production had begun. Both growth and pigment production were effectively stopped (TABLE III). In the second approach two sets of cultures were removed from the shaker before pigment production had begun; a control set was left on. The two sets removed were shaken by hand occasionally to prevent the development of surface growth. Analyses on one of these sets at the end of eleven days (TABLE III) revealed that even though consid-

TABLE III

EFFECT OF ANAEROBIOSIS ON GROWTH AND PIGMENT PRODUCTION
(*Aspergillus pseudoglaucus*. Czapek-Dox Medium)

Conditions of growth	Total growth Dry wt. per flask	Total pigment produced
10% Glycerol		
3 days O_2 + 9 days N_2	.028 g.	None
5 days O_2 + 7 days N_2	.037 g.	Trace only
12 days O_2	.137 g.	10% of growth
10% Glucose		
11 days SG*	.353	8% of growth
3 days SG + 8 days HSG*	.213	Trace only
3 days SG + 8 days HSG + 11 days SG	.352	12% of growth
10% Glycerol		
11 days SG	.250	14% of growth
3 days SG + 8 days HSG	.132	None
3 days SG + 8 days HSG + 11 days SG	.230	12% of growth

* SG = Submerged growth (produced on shaking machine).
HSG = Submerged growth (occasionally hand shaken).

erable growth had been obtained, under such poor oxygen tensions pigment production had been suppressed. This was true in studies with both glucose and glycerol as the carbon sources. At the end of eleven days the second set of hand shaken cultures was returned to conditions of vigorous aeration on the shaker and both growth and pigment production increased markedly during the next eleven days (TABLE III). While these data do not permit a definite conclusion as to whether growth is necessary for pigment production, oxidative metabolism is apparently essential.

Effects of iodoacetate on pigment production on various carbon sources

Experiments were extended to determine the effects of inhibitors on pigment production with carbon sources other than glucose and glycerol. FIG. 8 shows the effect of M/1000 iodoacetate on certain of these. This concentration was selected because at this level pigment production on glycerol is not inhibited. As can be seen, glucose, xylose and fructose cultures were markedly affected; sorbitol and mannitol cultures were affected little or not at all. Also markedly affected (though not indicated in the figure) were the growths on mannose, rhamnose and dulcitol. No growth could be obtained

in either submerged or surface conditions with either pyruvate, lactate, propyl alcohol or ethylene glycol as the sole carbon source in a basal Czapek-Dox solution. Results of further experiments to delimit the concentrations of iodacetate having an inhibitory effect on selected substrates are shown in TABLE IV. Interest in sorbitol and mannitol in connection with pigment production is further enhanced by the fact that the semi-quantitative results indicate that even somewhat higher concentrations of iodacetate are required to inhibit pigment production on these substrates than are necessary for pigment inhibition in glycerol growths.

Experiments with washed resting preparations to study possible pigment precursors

Some slight variability was occasionally noted in the results when attempts were made to determine end points of inhibition such as described. In one experiment, for example, M/750 iodoacetate

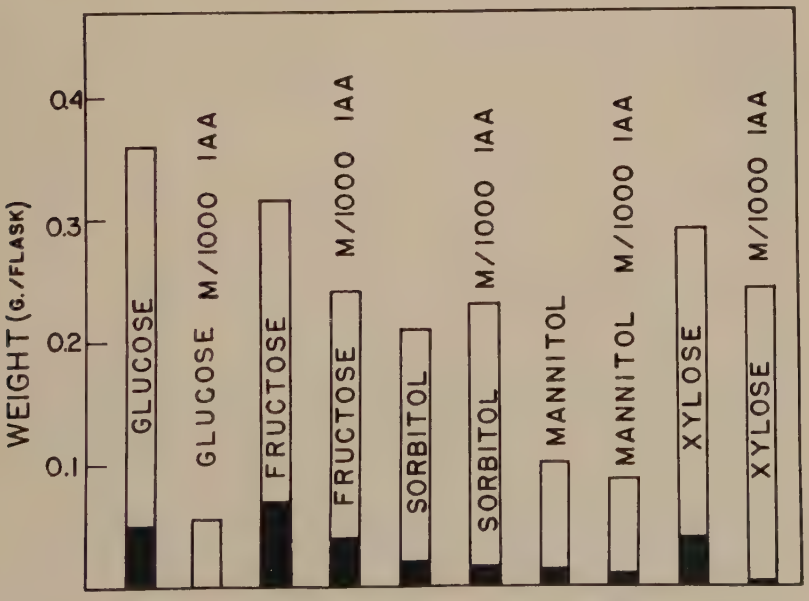

FIG. 8. Effect of iodoacetate (IAA) on growth and pigment production. Various carbon sources (concentrations 10%). *A. pseudoglaucus*. Submerged growths. Czapek-Dox medium. Ten days' incubation. Shaded area indicates pigment. Clear area indicates growth.

would inhibit pigment production on glycerol; in another, a tinge of color might develop and the ether extractions yield minute amounts of pigment. These differences have been ascribed to slight variations in the time of poisoning, small variations that might result in a slightly more vigorous growth, or which might allow the formation of some colorless precursor in the medium prior to the addition of the inhibitor. An experiment was designed to rule out, insofar as possible, the influence of components produced in the medium. For this purpose inoculations were made into media provided with a carbon source known to produce good growth. After three to

TABLE IV

EFFECT OF IODOACETATE ON PIGMENT PRODUCTION
(*Aspergillus pseudoglaucus*. Submerged Growth. Selected Carbon Sources.)
Grown 10 days at 24° C.

Concentration iodoacetate	Glucose	Glycerol	Sorbitol	Mannitol
M/100	−	−	−	−
M/250	−	−	+	−
M/500	−	−	+	±
M/750	−	+	+	+
M/1000	−	+	+	+
M/2000	−	+		
M/2500	−	+		
M/3000	−	+		
M/5000	−			
M/10,000	±			
M/20,000	+			

+ Definite pigment production.
− No definite pigment production.
± Trace of pigment production.

four days, when good colorless pellets had been obtained, the growth was removed, transferred to sterile centrifuge cups provided with sterile screw-top lids, and washed three times with solutions of the same osmotic concentration as those in which they were grown. The resulting mass of growth was diluted just sufficiently to enable it to be taken up, measured and dispersed by means of a sterile large-mouth pipette. Equivalent amounts of washed growth were then transferred to sterile flasks containing the substrates to be tested. When inhibitors were added they were incorporated at this time.

The data presented in TABLE V show the effect of various concentrations of iodoacetate on pigment production of washed glucose and glycerol growths resuspended in various carbon sources as well as in growth media, as indicated. The previous trends in the data are again confirmed. Pigment production on glucose is inhibited by concentrations far too weak to affect production on glycerol. Glycerol, sorbitol and mannitol behave as they did before. A striking observation is that the original growth medium was capable of supporting pigment production at unusually high inhibitor concentrations, either when the original medium contained glycerol or glucose.

TABLE V

EFFECT OF IODOACETATE ON PIGMENT PRODUCTION OF WASHED GLUCOSE AND GLYCEROL GROWTHS RESUSPENDED IN VARIOUS CARBON SOURCES AS INDICATED

(*Aspergillus pseudoglaucus*. Submerged Cultures)

Iodoacetate	Glucose growths					Glycerol growths				
	Old Medium*	Glucose	Glycerol	Sorbitol	Mannitol	Old Medium*	Glucose	Glycerol	Sorbitol	Mannitol
M/1000	−	−	−	−	−	+	−	−	−	−
M/2000	−	−	−	−	−	+	−	+	−	−
M/3000	±	−	±	±	−	+	−	+	−	−
M/5000	+	−	+	+	+	+	−	+	+	+
M/10,000	+	−	+	+	+		−			
M/20,000	+	+	+	+	+		+			

* Old Medium = the fluid in which the washed growth had grown.
+ Pigment produced; − No pigment produced; ± Trace of pigment production.

This is highly suggestive of the possibility that the growth medium contains probable precursors of the pigments. An examination of the data indicates that glucose growths replaced on old medium containing M/5000 iodoacetate produced pigment, while they did not when placed on fresh glucose medium containing M/10,000 iodoacetate. On the other hand glycerol growths placed on old glycerol medium could produce pigment at M/1000 iodoacetate as compared with no pigment production at M/10,000 iodoacetate when glycerol growths were placed on fresh glucose medium. If the organisms contained all essential precursors for the pigments this would not be the case. It appears therefore that the pigment

TABLE VI

INFLUENCE OF THE GROWTH MEDIUM IN RELATION TO THE EFFECT OF M/2000 IODOACETATE ON GROWTH AND PIGMENT PRODUCTION

(*Aspergillus pseudoglaucus*. Submerged Cultures. Washed Glycerol Growths)

New suspending medium	Pigment fifth day of growth	Pigment sixth day of growth	Pigment seventh day of growth	Total growth Dry weight grams (13 days)	Pigment per gram of growth (Dry wt. basis) (13 days)
Unwashed Control	−	+⁺	++⁺	0.370	.100
75 ml. OM (Old Medium)*	−	±	++	0.763	.080
50 ml. OM+25 ml. Glycerol	−	±	++	0.624	.042
25 ml. OM+50 ml. Glycerol	−	±	+⁺	0.676	.086
15 ml. OM+60 ml. Glycerol	−	−	+⁺	0.563	.048
5 ml. OM+70 ml. Glycerol	−	−	±	0.482	.046
0 ml. OM+75 ml. Glycerol	−	−	−	0.178	.000

* OM = Old Medium = the fluid in which the washed growths had grown.
− no pigment. + definite pigment. +++ most strongly pigmented.
± trace of pigment. ++ strongly pigmented.

precursor is associated with some soluble metabolic intermediate in the medium and is not concerned solely with components of the growth itself.

Additional evidence that substances in the original growth medium stimulate pigment production was obtained from experiments

TABLE VII

EFFECT OF VARIOUS CARBON SOURCES ON PIGMENT PRODUCTION THREE DAY WASHED GLUCOSE GROWTHS RESUSPENDED IN SUBSTRATE INDICATED

(*Aspergillus pseudoglaucus*. Submerged Cultures)

Substrate	Pigment on fourth day	Pigment on fifth day	Pigment on sixth day
5% Glycerol	−	+⁺	++
5% Sorbitol	−	+⁺	+⁺
5% Mannitol	−	+⁻	+⁻
5% Fructose	−	−	−
5% Glucose	−	−	−
5% Glucose + 5% Glycerol	−	+⁻	++
5% Glucose + 5% Sorbitol	−	+	++
5% Glucose + 5% Mannitol	−	+	+
5% Glucose + 5% Fructose	−	+⁻	+⁻
10% Sucrose	−	−	−
10% Glucose	−	±	+

− No pigment.
± Trace of pigment.
+ Definite pigment; +⁻ slightly less intense.
+⁺ Strongly pigmented but not as strong as ++.

of the type whose results are recorded in TABLE VI. Here glycerol growths were washed by centrifugation, as before, and resuspended in 75 ml. of glycerol medium. Pigment production on this medium was compared with that on various combinations of the new glycerol medium and the old glycerol medium in which the washed growths had been produced. The use of glycerol as a substrate results in considerable pigment; therefore these growths were partially poisoned with M/2000 iodoacetate to extend the time required for such pigment production as did occur. Both the qualitative and quantitative data show that growth and pigment production are directly related to the amount of old glycerol medium that is provided, sug-

TABLE VIII

PIGMENT PRODUCTION BY WASHED SUBMERGED GROWTH ON VARIOUS SUBSTRATES

Aspergillus pseudoglaucus Pigment per gram of growth (dry weight)

	Glycerol growths[1]	Glycerol growths[2]	Glucose growths[2]	Glucose growths[2]	Average of four experiments
Old Medium[3]	0.290	0.146	0.122	0.172	0.183
Glycerol	0.198	0.122	0.239	0.200	0.190
Mannitol	0.178	0.078	0.076	0.173	0.126
Sorbitol	0.184	0.047	0.084	0.134	0.112
Glucose	0.108	0.066	0.103	0.149	0.107

[1] Seven days on new substrate.
[2] Four days on new substrate.
[3] Old Medium—the fluid in which the washed growths had grown.

gesting that the old growth medium contains some precursor of the pigment or a factor that aids in its production.

Attempts to determine whether the factor might be one of the compounds previously studied were made by resuspending the washed growths on the various substrates suggested by the data. The results of such an experiment are recorded in TABLE VII. Glycerol, sorbitol and mannitol were found to be more effective in stimulating pigment production than other carbon sources tried. Such experiments have been placed on a quantitative basis and the results recorded in TABLE VIII. Here data have been obtained for two typical experiments using glycerol growths; two experiments using glucose growths, and the averages are given for the four attempts in which such an experiment was tried. Again glycerol,

mannitol, and sorbitol, in the order cited, seem the best pigment-producing substances found.

DISCUSSION

The experimental results above reported support certain suggestions as to the probable pathway by which strains of *Aspergillus* synthesize complex pigment molecules such as auroglaucin and flavoglaucin from glucose. The experiments with inhibitors suggest that reactions common to carbohydrate breakdown in yeast and animal tissue are also involved in pigment synthesis by members of the *A. glaucus* group. The results supporting this hypothesis are particularly striking since the concentration of inhibitor found effective in preventing pigment formation on glucose is of the same order as the concentration found effective in inhibiting glycolytic reactions with yeast and animal tissue preparations.

Further, pigment production from glycerol is not inhibited by concentrations of these inhibitors considerably higher than those which affect glucose metabolism. This suggests that once the sugar has reached the three carbon stage certain inhibitors of carbohydrate breakdown do not block pigment synthesis. In this connection it may be noted that pigment production from xylose, which is probably converted to a two and three carbon residue by a different route from that which glucose follows, is less affected by the inhibitors than is glucose.

The results strongly suggest that mannitol or sorbitol may be an intermediate in the synthesis of the pigments. This is particularly interesting since Yamasaki and Simomura (1937) have previously proposed that glycerol is a direct precursor of mannitol on the basis of experiments in which they were able to obtain 20–30% yields of mannitol from glycerol as a substrate using strains of *Aspergillus glaucus*. In our experiments mannitol and sorbitol often stimulated pigment production even more than glycerol, also suggesting the possibility of resynthesis of three carbon residues to six carbon residues. Furthermore, the fact that pigment production on mannitol could usually be carried on at higher concentrations of iodoacetate than those which inhibited pigment production on glycerol suggests that the three carbon residues are built into the larger residues

rather than the mannitol being degraded to the three carbon compound which is then utilized in pigment synthesis.

The data presented suggest the following tentative pathway for pigment synthesis. Glucose is converted by the usual pathway of carbohydrate dissimilation to the three carbon stage; the three carbon compound is then perhaps condensed to a six carbon residue such as mannitol which then is converted in some manner to the pigments. No conclusive evidence is available as to whether glycerol itself is the three carbon compound that is involved, but it is interesting that neither pyruvate nor lactate enhances pigment production.

The fact that oxygen is necessary for pigment production from both glucose and glycerol suggests that, assuming glycerol to be an intermediate in pigment production, oxygen is involved in the subsequent synthesis of the three carbon residues to the more complex molecule.

The possible role of mannitol as an intermediate in the formation of mold metabolic products is extremely attractive in view of the ubiquitous nature of the compound in mold metabolism as well as the fact that some of these organisms are known to produce extremely large quantities of it.

SUMMARY

(1) Auroglaucin and flavoglaucin are major end products of carbohydrate metabolism among members of the *Aspergillus glaucus* group. Striking quantities of these pigments are produced in both surface and submerged cultures.

(2) Even after considerable growth has developed, aerobic conditions are essential for pigment formation.

(3) In surface culture, organisms grown on glycerol, mannitol and xylose produced almost twice the amount of pigment per gram of mycelium as those grown on glucose. In submerged culture, organisms grown on glycerol produced even higher ratios of pigment per gram of mycelium than comparable glucose growths.

(4) In a basal Czapek-Dox solution no growth could be obtained in surface or submerged culture with either pyruvate, lactate, propyl alcohol or ethylene glycol as the sole carbon source.

(5) In submerged culture M/5000 and M/10,000 iodoacetate added the third day of growth inhibited both growth and pigment production on glucose while M/1000 iodoacetate inhibited neither growth nor pigment production on glycerol.

(6) In submerged culture M/250 sodium fluoride inhibited pigment production on glucose while concentrations as high as M/25 failed to inhibit production on glycerol.

(7) M/1000 iodoacetate markedly affected growth and pigment production on glucose, mannose, fructose, xylose, rhamnose and dulcitol; growth and pigment production on glycerol, mannitol and sorbitol was affected little or not at all.

(8) Pigment production by washed "resting preparations" on glucose is inhibited by concentrations of iodoacetate far too weak to inhibit production on glycerol, sorbitol and mannitol.

(9) Soluble intermediates in the growth medium may play a significant role in pigment production.

(10) The data presented suggest that the following tentative pathway may be involved in pigment synthesis by members of the *A. glaucus* group. Glucose is dissimilated by the usual route of carbohydrate dissimilation in yeast to the three carbon stage; the three carbon compound is then condensed to a six carbon residue such as mannitol, which is then converted in some yet undetermined manner to the pigments.

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ZONATION IN A PROLINELESS STRAIN OF NEUROSPORA [1]

WILLIAM H. BRANDT

(WITH 3 FIGURES)

There has been a considerable amount of investigation concerning the phenomenon of zoning, *i.e.*, the formation of regular differences in types of growth of fungi in culture, giving the appearance of concentric rings in petri dish cultures and regular bands in horizontal tubes. Most workers have attributed this to variations in the environment: Bisby (1925), Brown (1925), Gallemaerts (1912), Hedgecock (1906), Hall (1933), Hutchinson (1906), Knischewsky (1909), Molz (1906), Rahn (1912), and Snyder and Hansen (1941) found that alternation of light and dark caused zonation in their various test organisms. Temperature variation was shown to be the factor causing zonation in organisms tested by Bisby (1925), Ellis (1931), and Hall (1933). Munk (1912) found that fluctuations in humidity caused zonation of the fungi with which he was working. There has been some evidence, however, of the existence of another type of zonation, namely that which takes place in the absence of environmental fluctuations or which at least cannot be correlated with them. Hall (1933) states that: "Earlier workers have clearly recognized two types of successive zoning—first, a type which arises under uniform conditions of environment and, second, a type in which the zoning is correlated with a periodicity of the environment." Hedgecock (1906) mentions the first type and states: "This type has been observed by the writer in a number of species of fungi; among these is *Hypocrea*. Thomas Milburn mentions this type of ring formation in his studies of *Hypocrea rufa* and other fungi. In *Hypocrea* the concentric rings are formed, not daily, but in a much longer period

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of time." More recently, Brown (1925) noted something perhaps similar and said that some strains of *Fusarium* form one strong zone of spores which obviously has no relation to the periodicity of light. Stevens and Hall (1909) found that zonation of their test organism occurred either in constant light or constant dark and thought that this might be due to crowding of the mycelium.

The writer first noticed zonation in *Neurospora crassa* strain *21863,* while using it as a test organism in routine laboratory experiments. To his knowledge the phenomenon has not been previously reported in any strain of the species. At the time it was first observed, the tube technique of Ryan, Beadle and Tatum (1943) was being used and the zoning was manifested as alternated bands of dense and sparse formations of conidia. The writer thought it might be interesting to try to find the cause of zonation in this particular case; hence the following study was undertaken.

MATERIALS AND METHODS

Since the phenomenon was originally observed in tubes, and the organism grows at too rapid a rate to make petri dish culture practical, tubes were used throughout this investigation. In addition the tubes are generally easier to work with. The medium, also used throughout, was one devised by Gray (1946) and contained the following: 5 g. dextrose, 0.7 g. Difco yeast extract, 0.5 g. KH_2PO_4, and 2 g. agar per 100 ml. water. The liquid medium was pipetted into tubes which were plugged and placed in an autoclave. Changes take place in this medium during autoclaving, so it is desirable to keep the length of time in the autoclave about the same for each batch of medium in order that comparisons between experiments can be made. In this work a thirty minute autoclaving at fifteen pounds pressure (121° C.) was used; exceptions are so noted.

The portions of this research carried out under controlled conditions were done in a subterranean laboratory which was windowless, well insulated, and had a tight-fitting, refrigerator-type door. It was possible to maintain the desired temperatures within a range of $\pm \frac{1}{2}$° F. and relative humidity within a range of ± 2 per cent. A hygrothermograph recorded temperatures and relative humidities.

The source of light for this investigation was a single 60-watt

Westinghouse "daylight" bulb (blue coating) in a white metal shade, situated to furnish an intensity of illumination of about 15 to 25 foot candles. A dark box consisting of a box within a box was constructed of corrugated cardboard and placed immediately adjacent to the illuminated area. It had an opening four inches by four inches to allow air circulation so that the temperature in the box would not be too different from that outside. A maximum-minimum thermometer was kept inside the box during each experiment; it was found that the temperature inside the box was always within 1° F. of the temperature outside. The box was tested for possible light leaks by exposing photographic film (Eastman type B, press—Weston rating undetermined) inside the box for ten minutes while the light was turned on outside. When the film was developed, it showed no fogging, indicating there were no light leaks of sufficient intensity to cause a reaction of the emulsion.

The most accurate and practical method of recording results was deemed to be photographs of the culture-containing tubes taken at the end of each experiment. These were taken with light transmitted through the tubes as this method was found to give photographs of greater detail than if reflected light were used.

RESULTS

Investigation of Possible Zonation in Various *N. crassa* Strains

As zoning was observed in strain *21863*, it was thought that the phenomenon might occur in other strains of *N. crassa*. The following strains were therefore cultured in the laboratory under the same conditions in which the author first observed zonation in strain *21863*, namely, the diurnal variations in light, temperature and humidity to which the laboratory was ordinarily subjected. Note that some pigmented and some non-pigmented strains were used.

Strain	*Description*
21863	—pigmented, prolineless (unable to synthesize proline); this mutant is the test organism used in other portions of the study.
33757, 4637	—non-pigmented, leucineless.
15300	—an albino mutant.
34105	—pigmented, able to utilize ornithine, citrulline or arginine.
27947	—pigmented, able to utilize ornithine, citrulline or arginine.
4545	—pigmented, lysineless.

The light impinging upon cultures of these strains was of varying and unknown intensity and consisted of indirect sunlight and/or light from overhead lamps. A hygrothermograph recorded the temperature which varied from 79° F. to 84° F., and relative humidity which varied between 22 per cent and 38 per cent.

Several days later it was obvious that zonation had occurred only in the tubes containing strain *21863*.

Later, other strains of the ornithine cycle (Srb *et al.*, **1944**) were tested but none exhibited zoning. This does not imply that zoning would not occur under the proper conditions; a combination of inheritance factors similar to that responsible for zoning in strain *21863* might be present in one or more of these strains, its expression being masked by unfavorable (relative to zoning) environmental circumstances. The other strains tested were:

36703—argininеless
30300—citrulline-argininеless
33442—citrulline-argininеless
29997—ornithine-citrulline-argininеless

The remainder of the strains of this cycle as propounded by Srb, Fincham and Bonner (**1950**) were not tested since they were not immediately available.

Zonation Under Controlled Conditions

Since the previously mentioned zones appeared to correspond approximately to diurnal variations in light, an experiment was performed to study the effect of light on conidia formation. Fourteen tubes containing Gray's medium were inoculated with the test organism and placed in the controlled conditions room mentioned above. During this run, the temperature was 89° F. $\pm \frac{1}{2}$° F. and the relative humidity was 64 per cent $\pm$ 2 per cent. The lamp mentioned above was turned on so that germination and initial growth took place in the light. When the mycelial front had advanced a few millimeters, the position of the front was marked on each tube with a wax pencil and three tubes were placed in the dark box. Six hours later, the location of the mycelial front was again marked on the tubes remaining in the light and three more tubes were placed in the dark box. This was repeated with a

third and fourth set of three tubes each. Two tubes were left in the light to serve as controls. Ninety-six hours later, all tubes were removed from the dark box and the positions of mycelial fronts marked (FIG. 1). Zonation had taken place in all tubes which had been under the dark box and alternate bands were approximately equidistant in all tubes. It is evident from FIG. 1 that light inhibited zonation and that zonation occurred in each tube following the elapse of approximately the same time interval after being transferred to the dark. Once zonation occurred, however, the positions of the alternate bands appeared to be independent of the environment.

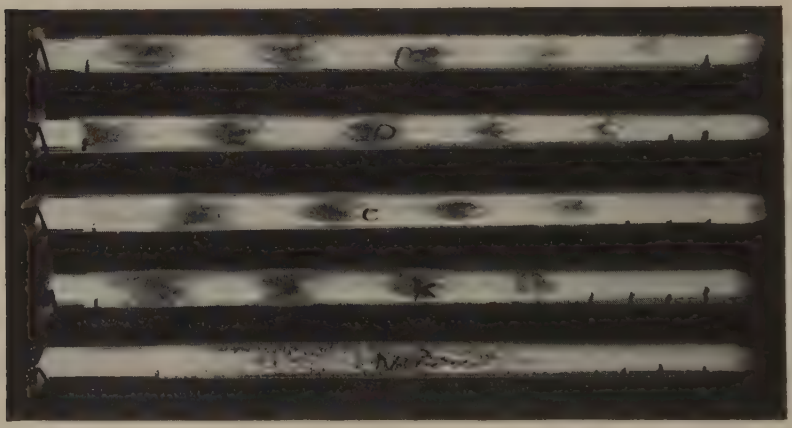

FIG. 1. Zonation under controlled conditions. Tube C was the first placed in the dark; tubes D, C and K were then placed in the dark 6, 12 and 18 hours respectively after C. Tube N was under continuous light. Note zoning in all tubes but N, and retarding of growth in tube N.

In these tubes a line connecting centers of zones was diagonal in relation to the tubes of different sets. Had fluctuations in any environmental factor been responsible for the zoning, a line connecting centers of zones would have been perpendicular to the tubes if they were placed parallel with all the first wax marks lined up.

Although in this experiment the conidia germinated in the light, and the tubes were subsequently placed in the dark, preliminary experiments had demonstrated that zonation will occur if germination of the conidia and subsequent growth of the mycelia occurs in total darkness.

Relation of Intensity of Illumination to Linear Growth

Beadle and Tatum (1945) stated that light intensity had no effect on the rate of linear growth of their test organisms, which were also strains of *N. crassa*. The author has noticed, however, that in addition to inhibiting zonation, continuous light seemed to slow down linear growth of strain *21863*. This experiment was performed to study the effect of light intensity on linear growth of the mycelium. Gray's medium was poured into three tubes, each about one meter long. Two of the tubes were placed under the same light source and moved during the course of the run so that the foremost 100 mm. of the mycelium was exposed to an intensity of illumination of 22 to 24 foot candles. The remaining tube was so placed that it received an intensity of illumination ranging from 0.04 foot candle at one end of the tube to 0.07 foot candle at the other end. Intensity of illumination was measured with a MacBeth Illuminometer, using the white disc placed at the same level as the tubes. The temperature range during the course of this run was 89° F. $\pm \frac{1}{2}$° F. and the relative humidity range was 66 per cent $\pm$ 2 per cent. The position of each mycelial front was marked daily and charted. While the light remained on, no zoning occurred in any of the tubes. A little more than sixteen days after the tubes were inoculated the light burned out and the tubes were left in the dark for three days, during which time no zoning took place in the tube which had been under the lower illumination, although slight zoning was apparent in the tubes which had been under higher illumination. In sixteen days in the light, the mycelium advanced 780 millimeters from the first mark in both tubes which had been exposed to the higher illumination while it advanced 887 millimeters in the tube exposed to the lower illumination.

Length of Dark Period Necessary to Permit Zonation

Since zoning occurred in continuous darkness, was inhibited by continuous light, but in preliminary experiments occurred under alternative light and dark periods each of 12 hours duration, it was inferred that there must be a minimum dark period which will permit zoning. It was further inferred that this minimum dark period must be within the range of 0–12 hours per 24-hour cycle.

Nine tubes, containing Gray's medium were inoculated with strain *21863* and placed in the dark in the controlled conditions room. During this run the temperature was 88.5° F. ± ½° F. and the relative humidity was 66.5 per cent ± 1.5 per cent. When the mycelial front had advanced a few millimeters, its position was marked on each tube. Three tubes were then placed under the dark box for a 2-hour period, three more for a 4-hour period and

FIG. 2. Length of dark period necessary to permit zonation. Tubes D, E, F were subjected to 2 hours of dark per day. Tubes G, H, I, to 4 hours of dark per day. Tubes A, B, C, to 6 hours of dark per day. When not in the dark, these tubes were subjected to light which had an intensity of illumination of 15 to 25 foot candles.

three more for a 6-hour period and then returned to the light. This procedure was repeated daily, one set of tubes thereby receiving 2 hours of darkness plus 22 hours of light, a second set 4 hours of darkness plus 20 hours of light, and a third 6 hours of darkness plus 18 hours of light. When the mycelial front had nearly reached the opposite end of the tubes, the results were recorded (FIG. 2). There was obvious zonation only in the tubes

receiving six hours of darkness. No obvious zonation occurred in either the set receiving two hours of darkness or the set receiving four hours of darkness, although there was perhaps a slight hint of zoning in the latter set, indicated more by slight difference in color and structure of the mycelium than by slight differences in its density. On the basis of these data the minimum dark period for obvious zonation under these conditions is of four to six hours duration.

Relations of Media to Zonation

A preliminary run made in the earlier stages of the investigation had shown that a sugar concentration of 5 per cent was better as far as zoning was concerned than 0.5 per cent or 10 per cent, so 5 per cent was the concentration used throughout the work. The effect of various kinds of media on zonation was also investigated. These media were: prune agar, nutrient agar, lima bean agar, corn meal agar, brain-veal agar, beef-lactose agar (all Difco products), potato-dextrose agar (200 g. potato tuber, 20 g. dextrose, 17 g. agar, 1 liter of water), and two lots of Gray's medium, one autoclaved for 30 minutes and the second autoclaved for 90 minutes. The clue that autoclaving this medium more than 30 minutes had some effect upon its composition was provided when tubes were inadvertently left longer than this period in the autoclave. When the tubes were finally removed, it was observed that a decided color change in the medium had occurred. Upon cooling, the agar did not form as firm a gel as that which was autoclaved for only 30 minutes. This medium was included in the experiment to ascertain whether or not any changes had occurred in the medium which would significantly affect zonation. Three tubes of each medium were inoculated with strain *21863* and placed in the controlled conditions room with the light on.

When the mycelial front had advanced a few millimeters, its position was marked on the tubes and the light was turned out. Five days later the position of the mycelial front was again marked and the results were recorded (FIG. 3). Obvious zonation occurred only in the tubes containing Gray's medium—the zones being a little better defined on the medium which had been auto-

FIG. 3. Relations of media to zoning. On the above tubes, the labels denote the following: CM—corn meal agar; P—prune agar; LB—lima bean agar; G3—Gray's medium autoclaved 30 minutes; G9—Gray's medium autoclaved 90 minutes; PD—potato-dextrose agar; BV—brain veal agar; BL—beef lactose agar; Nu—nutrient agar. The wax pencil marks on the left denote the position of the mycelial front.

claved 90 minutes. Zonation on other media was poor or not present at all.

The Relation of Proline to Growth of Strain *21863*

In order to be sure that strain *21863* was still prolineless and had not lost this character in culture and to see what effect, if any, different concentrations of proline might have on zonation and linear growth, a series of tubes was set up containing a minimal medium plus varying concentrations of proline. This medium is similar to that described by Beadle and Tatum (**1945**) in their work on nutritional requirements.

The writer arbitrarily selected the following amounts of proline: 0 mg., 1 mg., 2 mg., and 4 mg. per 100 cc. medium. Three tubes

were half-filled with each of these media and inoculated with strain *21863* and placed in a dark room containing no temperature or humidity control equipment. However, a hygrothermograph was placed immediately adjacent to the tubes and recorded a temperature range of 83–87° F. and a relative humidity range of 24–32 per cent.

Nine days later, the tubes were observed, and the results recorded. In the tubes containing no added proline, germination and growth was limited to small tufts, none of which exceeded 5 mm. diameter. In the other tubes, containing various concentrations of added proline, the mycelium had grown about 250 mm. in most cases. The rate of linear growth seemed to be about the same regardless of the concentration of proline but the amount of mycelium appeared to increase directly with proline concentration.

There was no zoning in any of these tubes, the growth appearing irregular. There were, however, in the tubes containing 4 mg. proline per 100 cc. medium, scattered patches which had the general appearance of the type of growth in the dense area of a zone. In general the growth was different from the type of growth obtained under continuous light. It appears from these observations that there was some ingredient which is required for zoning lacking from the medium or perhaps it was not present in the proper quantity. It is also possible that some constituent of the medium inhibited zonation.

DISCUSSION

Zonation in strain *21863* seems to be a different sort of phenomenon than that described by most previous investigators, although it is possible that it is similar to that described by Hedgecock (**1906**), Milburn (**1904**) and Brown (**1925**). It is doubtful that it is related to the type discussed by Stevens and Hall (**1909**), since zoning in their test organism seemed to be due to alternate concentric areas of dense and sparse mycelium, whereas in strain *21863* the zoned appearance was due to alternate bands of dense and sparse conidia. The writer was unable to find an instance where either Hedgecock, Milburn or Brown carried out controlled experiments which definitely showed that their particular test organism zoned under relatively constant conditions. This would

have been reasonably easy to show had the tube technique been known. This technique offers excellent possibilities of finding the exact chemical and physical processes which result in zoning in strain *21863*.

Most of the experiments which were completed in this investigation are primarily exploratory and the openings for additional experimentation are abundant. One striking possibility for further investigation is that perhaps this phenomenon is related to the ornithine cycle, or if not, it may prove to be a useful character in the genetic phase of this investigation.

Although the writer feels premature in proposing a possible mechanism for zoning in strain *21863*, the following hypothesis is offered in an attempt to relate data available at this stage of investigation.

Consider a tube recently inoculated with strain *21863* which has been under light until the colony reaches whatever size and physiological state is necessary for zoning to occur. When the light is turned out, a certain series of events take place, namely: the colony continues growing down the tube for about six hours (89° F.) with no noticeable increase in its density; then a very apparent dense area of conidia and aerial hyphae is formed for several hours; this type of growth then ceases and the former sparse type resumes for several hours until the dense type starts again, etc. Suppose that when the light is turned out, a chain of reactions is able to go to completion which results in zoning; since zoning doesn't occur when the light is left on, at least one substance produced in this chain must be light labile, and the chain is thus blocked by the light.

Postulate further the production and accumulation in the course of chain "α" of a light stable substance D which is necessary for the fabrication of the dense zone. That this substance is light stable is demonstrated by the observed fact that light applied just after the dense zone begins to form doesn't inhibit the formation of the rest of the zone. The zone will continue to form in the light as long as 6 to 10 hours after the application of light.

However, even if no light is applied, the formation of the dense region eventually ceases. To explain this phenomenon a substance "X" is hypothesized which is produced by a side reaction during

the preceding series of events which inhibits conidia formation when present in sufficient concentration—further, it inhibits the resumption of the chain of reactions "α" until the mycelium grows beyond its influence. Then, if no light is present, chain "α" starts again and in about six more hours, the dense zone is started once more.

Assume that the size of the dense area is proportional to the amount of D synthesized; then if light is applied soon after a small amount of D is produced, the dense area should not be very dense and not very obvious. This is the case where tubes were subjected to only four hours of darkness—when they got six hours, the zones were quite obvious. No zones could be detected where the tubes received 2 hours of darkness and it is inferred that the reactions resulting in D had not had time to go to completion before light destroyed the labile precursor, while in the tubes receiving 4 hours of darkness, the reaction apparently had produced a small amount of the stable D before the light inhibited its further production. From these observations, it is inferred that under the conditions of this investigation, the reactions producing "D" were between 2 and 4 hours long, seemingly closer to 4 hours than two.

Since the first conidia of the dense zone seemed to appear about six hours after the inhibitory effect of either light or factor "X" were removed, it is inferred that the reactions starting with "D" as a substrate go to completion with the formation of structures of the dense zone in about 2 (perhaps somewhat more) hours. That is, 6 hours minus the time required to produce D.

If the light is not turned off at the time indicated at the beginning of this discussion, no zonation will take place; that is, there is no alternate formation of dense and sparse zones. However, under continuous illumination, conidia and aerial hyphae do begin to form in abundance approximately 18 (± 6) hours after the colony is physiologically capable of forming a dense zone in the dark. The chain of reactions resulting in non-zonation in continuous light will be referred to as chain β. Since a different structure results in continuous light, it is inferred that a different process must be involved, at least in part. It is apparent in FIG. 2 that there is a considerable amount of rather dense growth between the so-called "dense areas." It appears that chain β is taking place in the

tubes which received 2 hours of dark and that perhaps it is still taking place to some extent in the other tubes and that the only difference in chains of reaction α and β is the production of "X." The writer would be inclined toward this point of view were it not for the observation that the appearance of dense growth produced by α is much different from that of chain β. Which is the valid point of view could perhaps be decided by microscopic examination of hyphae and spore structure but this was impossible with the type of tube used.

If the above hypothesis is correct, manipulation of the zones should be possible. Preliminary tests indicate this to be the case. Application of light to the colony before the inhibitory effect of "X" had dissipated prevented dense areas from forming after the usual interval of sparse growth. Regular zoning was resumed when the light was removed after a 48-hour period of sparse growth. In another preliminary test, the length of dense and sparse zones was cut down by light manipulation so that five dense zones were formed in the test colonies in the same time that four dense zones were formed in the controls. However, neither of these tests was performed in a critical manner, and the results should be interpreted as indicatory only.

SUMMARY

1. *Neurospora crassa* strain *21863* zoned under certain conditions.

2. Nine other strains of the same species did not zone under the conditions which permitted zoning in strain *21863*.

3. Under constant environmental conditions, strain *21863* zoned in continuous darkness.

4. Under constant environmental conditions, strain *21863* did not zone in continuous light.

5. The minimum dark period per 24-hour cycle which permitted obvious zoning was of 4 to 6 hours duration.

6. The medium on which zoning was most distinct (of the media tested by the writer) was Gray's medium autoclaved for 90 minutes, and was almost as distinct on the same type of medium autoclaved for 30 minutes.

7. Continuous light resulted in a reduction in rate of linear growth; 22–24 foot candles retarded linear growth more than 0.04–0.07 foot candles.

8. Intensity of illumination as low as 0.04–0.07 foot candles inhibited zonation when supplied continuously at 89° F. and relative humidity 66 per cent.

The writer wishes to express his sincere appreciation to Dr. W. D. Gray for his suggestions, technical assistance, and for crictically reading this manuscript.

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PHAGE PROBLEMS IN THE STREPTOMYCIN FERMENTATION

FERNANDO CARVAJAL [1]

(WITH 3 FIGURES)

Since 1915 and 1917 when Twort (12) and d'Herelle (5) discovered the phenomenon of bacteriophagy, interest has been focused on the nature of the phage, its reproduction and the mechanism of its action upon the host cell. The phages of bacteria and Actinomycetes have most of the characteristics usually attributed to the animal and plant viruses.

Since the discovery of the phage of *Streptomyces griseus* in 1947 by Saudek and Colingsworth (10), similar outbreaks of the phage were reported from other sections of this country and later on from other countries. These outbreaks have resulted in considerable loss of streptomycin production to the industry. The phage of *Streptomyces griseus* has been called an *Actinophage,* though it should more properly be named a *Mycophage* due to the nature of *Streptomyces griseus,* which is a typical fungus (2, 3). For simplicity in the present publication it will be referred to as phage of *Streptomyces griseus.* The term *Mycophagy* indicates the phenomenon of host cell disintegration by the action of the phage.

Since 1945 we have been studying fermentations with *Streptomyces griseus* for the production of streptomycin. However, it was not until March, 1952, that we encountered any phage outbreak. This led us to a more detailed study of the whole phage problem in streptomycin fermentation. The sources of the phage used in all of the work reported in this communication were infected tanks at our commercial plant. The *Streptomyces griseus* used was the

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culture *U-203,* a mutant produced by ultraviolet light irradiation of the original culture *SL-842* which was isolated from Ohio River mud in our laboratories in March, 1945 (**4**).

PHAGE IN THE ACTINOMYCETES WITH SPECIAL REFERENCE TO THE MAIN PUBLICATIONS ON *STREPTOMYCES GRISEUS* PHAGE

In 1936 Wiebols and Wieringa (**17**) were the first clearly to prove the existence of phage in the group Actinomycetes. They called this phenomenon *Microbiophagy.* They found phages in species of *Streptomyces,* including *S. scabies* obtained from infected potatoes. They also found phages from the pathogenic *Actinomyces bovis* and *Nocardia farcinica.* They produced the typical plaques and lysis due to the phage action upon the various species of Actinomycetes comparable to those caused by bacteriophages upon bacteria. They also reported a phage with the ability of lysing more than one species of Actinomycetes, *i.e.,* a polyvalent phage.

Saudek and Colingsworth (**10**) were the first to report, in 1947, the discovery of a phage for streptomycin-producing strains of *Streptomyces griseus* in their fermentations. The phage propagated rapidly in young cultures of *Streptomyces griseus,* bringing about the lysis of the organism. Cultures resistant to the phage were isolated for streptomycin production. When the phage was added to a fermentation with the sensitive strain of *Streptomyces griseus,* production of streptomycin was partially or completely prevented. The plaque method was used to measure the amount of phage particles present in the broth.

In 1947 Woodruff *et al.* (**18**) isolated a phage in laboratory stationary liquid cultures of *Streptomyces griseus* in flasks which were exposed to laboratory air for 24 hours. They reported that the strains of *Streptomyces griseus* which do not produce streptomycin were resistant to the action of the phage and that only the streptomycin-producing strains were sensitive to the phage. They also reported that six additional species of *Streptomyces* were not affected by the phage. Studying the morphology of the phage in the electron microscope, they found the phage to resemble *Escherichia coli* phage. They described it as follows: the majority of the heads were symmetrically spherical, 0.05 μ in diameter; tail

bent, long, relatively thick, about $0.15 \times 0.015\,\mu$. Many phage particles consisted of two bodies and a few were tetrads.

In 1947, Reilly, Harris and Waksman (**9**) fully confirmed the previous findings of Saudek and Colingsworth (**10**) and Woodruff *et al.* (**18**). Reilly and coworkers demonstrated that the phage attacks only the streptomycin-producing strains of *Streptomyces griseus* and it has no effect on other streptomycin-producing organisms such as *S. bikiniensis*. Other species of *Streptomyces* as well as the other genera of Actinomycetes were not affected by the *Streptomyces griseus* phage. The phage multiplies only in living cells and not on the heat-killed organisms. The phage has an optimum temperature for multiplication of 28° C and it does not multiply at 37° C or above. It can withstand 75° C for one hour but it is killed at 100° C after 10 minutes. It can be stored at 6° C without loss of activity, but at 28° C or higher it soon loses its activity proportionately to the rise in temperature. The nature of the medium also affects the rate of destruction of phage activity.

Waksman, Reilly and Harris (**14**) used the phage of *Streptomyces griseus* for identifying streptomycin-producing strains of *S. griseus* especially in the fresh isolation of strains from soils.

In 1950 Koerber *et al.* (**7**) reported two different types of *Streptomyces griseus* phages. They distinguished these types by the host specificity, plaque morphology and size of particles. The larger size phage produced the smaller type of plaque.

Umezawa *et al.* (**13**) reported in 1950 their observations on the *Streptomyces griseus* phage. They found that the streptomycin yield increased for about 10 hours after almost all mycelium was lysed and the broth had become almost clear. Furthermore glucose was rapidly consumed after the broth had become clear. Among 28 streptomycin-producing strains of *Streptomyces griseus*, 17 strains were lysed by the phage. The phage was not effective against grisein-producing *Streptomyces griseus* strains, indicating that the phage was strain-specific. Therefore, they stated that the use of streptomycin-dependent bacteria for the purpose of identifying the streptomycin-producing strains of *Streptomyces griseus* was superior to the use of an Actinophage.

Perlman *et al.* (**8**) reported that the multiplication of the bac-

teriophages of *Streptomyces griseus* may be limited by the addition of substances such as citrate, oxalate and phytate capable of sequestering calcium in the media before infection of the host cells takes place. These substances inhibited phage multiplication in submerged culture fermentations without adversely affecting streptomycin production. The effectiveness of the inhibition of bacteriophage multiplication may be reversed by addition of metallic ions forming complexes with these sequestering agents.

Watson reported (**15**) that magnesium and manganese ions supported the growth of the *Streptomyces griseus* phage S-1, being required as absorption co-factors. The phage multiplication was completely inhibited by certain concentrations of the alkaline cations, sodium, potassium and ammonium. This inhibition was attributed to an antagonism of the required metals by the alkaline salts.

Abe, Shiotsu and Endo (**1**) in 1952 reported their studies on the phage of *S. griseus* S-1. They studied the morphology of the phage with the electron microscope and reported it as being of a tadpole or spermatozoidal shape with a cocoon-like head of 120–130 mμ in length, 70–80 mμ in width and a tail of 160–200 mμ in length and 30 mμ in width and called this *N-1 phage*. This Japanese strain of phage is much larger than the strain reported by Woodruff (**18**).

In 1952 Gilmour (**6**) isolated from soil a phage designated as *514-3* which was able to attack a large group of *Streptomyces* species. It was pointed out that, instead of a single kind of phage, several were probably present, as evidenced by the different morphology of the phage particles themselves and by the varied action of single plaque cultures upon the various Streptomyces species.

PHAGE OUTBREAK IN THE S. GRISEUS FERMENTATION

In March, 1952, it was observed that the mycelial growth of *S. griseus* in some fermentation tanks became suddenly and totally autolyzed. As soon as the presence of phage was suspected, it was confirmed by testing the Seitz filtrates of the broth. The end results of the phage contamination in the fermentation tanks were usually a characteristic increased fluidity, diminution or ab-

sence of mycelial growth, little or no streptomycin, the presence of a dark brown soluble pigment and the presence of the phage. The dark pigment production is due to the strong enzymatic reactions which accompany the autolytic destruction of *S. griseus* by the phage action.

Careful investigation revealed that in none of our stock culture collection of *S. griseus* was the phage present. In spite of strict sterility control measures, the infection must have been introduced in some subsequent operation during the scale-up to plant fermentation.

Through the development of phage immune strains not only was the phage eliminated, but also the streptomycin yields were significantly increased by approximately 17 per cent. Out of 111 tanks which were sampled during a three week period and tested for phage, 74 were found to be infected. Among these 74, some were also contaminated with bacteria. The remaining 47 were free of phage. All the above 111 Seitz filtrate samples were preserved at 3–5° C for further work. The fermentation samples were taken at times varying from 12–72 hours of fermentation. The amount of phage particles in the broth of these 74 infected tanks varied from 100 to approximately a trillion phage particles per ml. of the broth. This indicated that the tanks were infected at different times in the fermentation period. In testing the tank broth samples, two methods were used: 1) the whole broth was streaked on the surface of agar medium to determine type of growth of *S. griseus* and the presence or absence of contaminants such as bacteria and phage, and 2) the Seitz filtrate was tested for the presence of phage.

The first infected tank tested contained 50 billion phage particles per ml. of broth after fermenting for 42 hours at 25° C. The phage obtained from this broth was named phage No. *1*. Phages No. *1* and No. *39* were selected for experimental use throughout this work because of their highly effective mycophagy upon the *S. griseus* cultures. Phage No. *39* was obtained from broth from a 28-hour old fermentation tank.

These phages were multiplied by inoculating shake flask cultures containing the spores or young mycelial growth (24–48 hours

old) of a phage-sensitive strain of *S. griseus* with a suspension containing a few phage particles.

MATERIALS AND METHODS FOR PHAGE DETECTION

1. *Filtration of Broth Samples:* Broth samples were taken from fermentation tanks or from stationary or agitated cultures. It was necessary to separate the mycelial growth and the rough particles of media from the fermented broth by filtering first through filter paper or through cotton held between two layers of cheese cloth. The clear filtrate was then passed through the Seitz filter in order to obtain a sterile filtrate containing any phage that may have been present. These filtrations were performed with a Seitz filter open on top and connected through a well-tightened rubber stopper to a side arm pyrex flask, connected to a vacuum pump. The flask, with the filter in place, was sterilized in the autoclave previous to each filtration.

For convenience and practicability, the Seitz filters used throughout this work were the standard laboratory models commonly known as Seitz Combination Uhlenhuth-Manteufel, Laboratory Model Size 6. Once the sterile filtrates were obtained, they were tested by streaking them on double layer seed plates.

2. *Double Layer Seed Plates:* Agar plates were prepared with the following media: A or B, used interchangeably as a nutrient medium throughout this work, varying only the amount of agar to give the required percentages, or omitting it, when used as liquid media.

	Medium A	Medium B
Water	1 liter	1 liter
Dextrose	10 grams	10 grams
Difco yeast extract	10 grams	20 grams
K_2HPO_4	0.5 grams	0.5 grams
$MgSO_4 \cdot 7H_2O$		0.5 grams
Agar	15 grams	15 grams
	pH 6.8–7.0	

The agar plates (100 × 20 mm) contained a base layer of 20 ml of medium and after it solidified, a top seed layer of 2 ml was added which contained the spores of *Streptomyces griseus* (sensitive to phage), from a slant or agar plate culture. The spore suspension was added to the medium at 42–45° C just before pouring. The

bottom layer contained 2 per cent agar and the top or seed layer contained 1 per cent agar. The addition of 100 units of streptomycin per ml of medium in both layers may be used to prevent the growth of bacterial contaminants. The streptomycin was added to the upper layer at the same time as the *S. griseus* spores. This type of plate was also excellent for detecting the presence of phage in the air as described below. Before streaking the plates with the phage filtrates, the surface of the seed layer should be hardened and well-dried. Each sample can be used in three strengths:

1. undiluted filtrate
2. dilution of 1 : 10,000
3. dilution of 1 : 100,000,000

The plates were streaked with a wire loop which held 0.01 ml of sample. Three parallel streaks were made per plate in the above dilutions, care being taken not to touch the edge of the plate with the loop. The cultures were then incubated at 28° C for 1–5 days; results were observed within 16–24 hours of incubation.

One of the dilution streaks usually showed a few clear plaques. In these cases it was easy to make a rough estimate of phage particles per ml of the original filtrate. See FIG. 1.

It was interesting to note that numerous spores of *S. griseus* as well as some mycelium were often found in the areas and plaques where there was no visible growth. These spores, when transferred to fresh media, failed to germinate, implying that they were either dead cells or would be soon because of their inability to grow and multiply. These spores, when observed under the microscope, were found to be normal in size and morphology.

3. *Double Layer Phage Assay Plates:* These plates were prepared in the same way as the double layer seed plates described above, with only one difference, viz., the top layer was poured with medium containing the proper dilution of the phage in addition to the spores of *S. griseus* phage-sensitive strain. This method of using two layers of agar always gave clear-cut plaques due to the thinness and the softness of the top seed layer which was ideal for mycophagy. See plate 1 in FIG. 1. This method was superior to that in which the phage and the sensitive organisms were

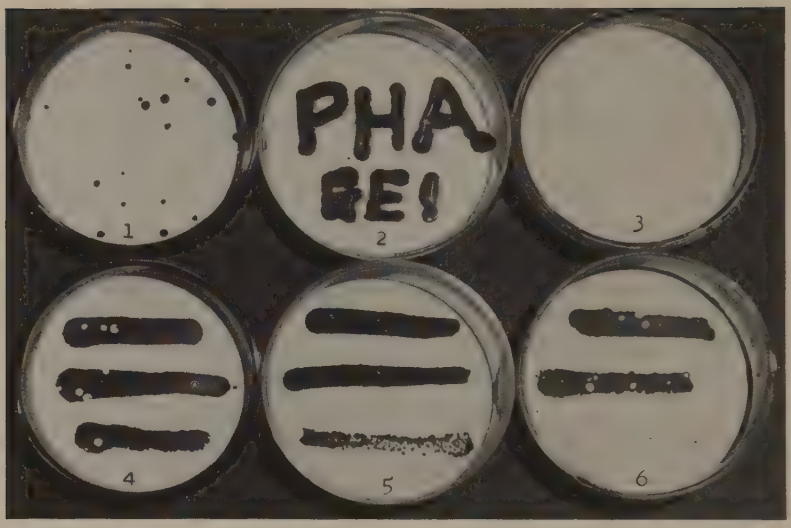

FIG. 1. Phage action upon *S. griseus U-203*. Cultures 10 days old at 25° C. Plate 1: Double layer phage assay plate showing typical phage plaques as clear circular areas without any growth of *S. griseus* while the rest has a nice heavy growth of *S. griseus*. Plate 2: Shows the name PHAGE which was written with the aid of a wire loop on the surface of a double layer seed plate with a phage filtrate which contained 10 billion phage particles per ml. The name PHAGE is shown as clear and transparent letters without any growth among the heavy growth and sporulation of *S. griseus*. Plate 3: Control agar plate without any phage showing heavy growth and sporulation of *S. griseus U-203*. Plates 4, 5, and 6: Show the phage action from 3 different filtrates (3 different phage contaminated tanks) upon *S. griseus U-203*, in double layer seed plates. These 3 plates show clear streaks (no growth) from left to right through the *S. griseus* growth. Each streak contains 0.01 ml at the given dilution of the phage. The top clear streak in each plate is the undiluted filtrate; the second or middle streak is the filtrate diluted in distilled sterile water 1:10,000 and the 3rd or bottom streak is the filtrate diluted 1:100,000,000.

Note: The plaques, the moth-eaten areas, and the isolated phage-resistant *S. griseus* colonies in the phaged streaks show the typical phage action upon its host *S. griseus*. The filtrates on plates 4 and 6 gave more resistant colonies than the filtrate on plate 5.

poured onto plates in a single layer of about 15–30 ml of medium containing two per cent agar inasmuch as the plaques overlapped and resistant colonies lying between the plaques in a vertical plane obscured the results.

The phage plaques, which were the clear areas with no growth

of *S. griseus* but containing the phage, varied in size and shape (see FIG. 1, plate 1) appearing more or less round to the naked eye.

4. *Single Layer Phage Plates:* These plates were prepared by incorporating the phage into the agar medium at 42–45° C, pouring the plates and allowing them to harden. For best results, medium containing one per cent agar was used. The agar medium contained from one million to one billion phage particles per ml. The single layer phage plates were used as soon as possible to avoid drying out and consequent loss of mycophagy.

This type of plate was employed for testing cultures of *S. griseus* for phage sensitivity and phage resistance. A suspension of spores or growth of the culture was streaked on the surface and then the plates were incubated at 28° C. Results were obtained within 1–5 days.

When testing strains of *S. griseus* which produced little or no streptomycin, care was taken to avoid the presence of streptomycin in the phage filtrates because of the inhibitory action upon the growth of these inactive strains.

5. *Isolation of Phage from Air:* It was a very simple process to determine the presence of phage particles in the air of rooms, laboratories, tank fermentation rooms or out of doors. Double layer seed plates were exposed to the air for 1, 3, 5, 20, 30 minutes and 1 hour, etc., and then incubated at 28° C. Definite and clear results were quickly obtained within 1–3 days. For instance, during the short-lived phage infestation at the plant, by exposing plates to the air for 3 minutes in the fermentation tank room, 500–1000 phage plaques developed per plate. After two months no phage was obtained from the air even after long exposure, due to the employment of phage-immune strains of *S. griseus*, institution of stricter sanitation control, and the temperatures of 28–33° C usually prevailing in the fermentation rooms.

The number of plaques developing on the exposed plates was proportional to the degree of severity of the phage infestation in a given fermentation area. Repeated exposure of plates over several days or weeks gave us an idea of the effectiveness of the sanitation program. The following faulty practices led to the air becoming heavily loaded with phage particles: 1, emptying infected

tanks without first sterilizing them; 2, permitting exhaust air of contaminated tanks to be vented inside the room without the use of a trap, and 3, blowing steam through sampling valves of infected tanks after samples had been taken. The first blast of steam through the valve threw into the air a fine mist containing millions of living phage particles. The object of the use of the steam was to keep the valves clean and sterile. However, to guard against air contamination, some arrangement such as trapping should be employed. The above discussion applied equally well to other contaminants such as bacteria.

6. *Isolation of Phage from Soil:* When a particular soil was to be examined for the presence of phage, or when phage was to be isolated from soils for a particular culture the following simple procedure was followed.

Ten grams of fresh soil (or mud) were added to 100 ml of broth medium A or B which contained 100 mcg of streptomycin per ml of medium (to avoid bacterial growth), and a heavy spore suspension of *S. griseus* for which a phage was desired. The culture was incubated from 1–3 days at 28° C, the broth was Seitz filtered and the sterile filtrate was tested using double layer seed plates. Agitated cultures 24–48 hours old in medium A, B, or C on which there was a nice young mycelial growth could also be inoculated with the soil plus 100 mcg of streptomycin per ml as an optional addition. The procedure for testing followed as above.

This method can be used for testing the presence of phage not only in soil samples but in other materials such as rough media, nutrients like soybean meal, various proteinaceous and carbohydrate substances, corn steep liquor, etc.

MYCOPHAGY

The phenomenon of the mechanism of action which determines the lysis of the spores and mycelium of the fungus *S. griseus* and the formation of plaques, is dependent on the availability of free water in the medium, which helps in the diffusion of the phage and in the penetration and lysis of the cells by the phage. The water supply in liquid cultures is ideal for mycophagy, bringing the phage in contact with all the host cells. In a semi-solid medium which is

too hard, due to the presence of more than two per cent agar, the mycophagy is very much reduced or absent. Therefore it was essential to use a thinner layer and a softer agar, as for instance one per cent agar, where the water along with the phage diffused readily through the layer of medium, thus bringing the phage in contact with the host cells. The presence of free water contained in soft agar not only helped in the transportation and diffusion of the phage but also in the penetration and disintegration of the *S. griscus* cells by the phage, bringing about a more complete lysis and consequently larger and clearer plaques.

This mechanism of action can easily be demonstrated by the following examples: a) A phage filtrate was streaked at various dilutions on the surface of a double layer seed plate. The top layer of one per cent agar was seeded with spores of a sensitive strain of *S. griseus*. The phage then diffused into and through the top layer, coming into contact with the *S. griseus* spores and mycelium and penetrating them. The consequent complete lysis appeared as very clear streaks and plaques as can be clearly seen in FIG. 1. This example indicated that the available free water helped in the transportation of the phage and in the penetration and lysis of the host cells by the phage. b) From a million to a billion phage particles per ml of medium were mixed into the medium containing *S. griseus* spores before pouring plates or making slants. When adding the phage and the spores the agar is about 42–45° C. After pouring plates and making slants the cultures were incubated at 28° C for one to two weeks time. As a result, some colonies of *S. griseus* appeared to be distributed throughout the agar. Among the agar plates we found many which had numerous colonies growing around the edges of the surface of the agar where the agar was in contact with the glass walls of the petri plate. The majority of these colonies were still phage sensitive while the other colonies away from the edges of the plate were phage resistant. Among the agar slants, practically all showed heavy growth (largely phage sensitive) on the dry top portion of the slant while only a few resistant colonies appeared in the rest of the agar slant. In this example the spores and phage were brought into contact by their initial mixture in the fresh medium. c) Solidified agar slants which contained the phage

were inoculated on the surface with a heavy spore suspension of *S. griseus*. The same growth observations were noted as in example "b" above. This is illustrated by tubes 1, 2, and 3 of FIG. 3, where *S. griseus* is growing nicely on the top, drier sections of the slants, while in the center and lower portions, where moisture is more available, the mycophagy is evidenced by the growth of only a few resistant colonies of *S. griseus*. The majority of those *S. griseus* colonies which grew in the drier portions of the slant or plate were still phage sensitive, the phage being unable to bring about the phenomenon of mycophagy due mainly to lack of water.

The same phenomenon of mycophagy using *S. griseus* phage sensitive strains was also exemplified by using another fungus, *S. olivaceus* NRRL No. *B-1125*, in the presence of phage.

TESTING OF VARIOUS ACTINOMYCETES FOR PHAGE SENSITIVITY

Cultures of various species representing the five genera of the Order Actinomycetales (*Mycobacterium*, *Nocardia*, *Actinomyces*, *Streptomyces*, and *Micromonospora*) were tested for phage sensitivity, using the single layer phage plate technique.

A total of 84 cultures of *Streptomyces griseus* were used in these tests. Of these cultures 76 were isolated in our laboratories from various soils, six were kindly supplied by Dr. S. A. Waksman, Nos. *4*, *9*, *19*, *3326*, *3478*, and *3510*, and two by the Northern Regional Research Laboratory, NRRL No. *B-148* (Carvajal's *SL-2060*) and NRRL No. *B-1075*. The 84 cultures represented 66 streptomycin producers and 18 non-producers.

Of the 66 streptomycin-producing cultures only five were resistant, while the rest were sensitive to the action of the phage. Among these phage-resistant cultures, a lyophilized culture *S. griseus SL-841*, which was a good streptomycin producer, was isolated from Ohio River mud in March 1945 (4) and kept preserved in a lyophilized state since that date. It was interesting to notice that culture *S. griseus SL-842*, which was also a streptomycin producer and was isolated at the same time and from the same sample of soil, was very sensitive to the phage action.

All of the 18 non-streptomycin-producing cultures were not affected by the phage with the exception of the two grisein pro-

ducers, Waksman's *3478* and *3510*. Culture *3478* was more sensitive to the action of the phage than *3510*.

When a culture of *S. griseus* RH-*674*, a streptomycin producer, grew on an agar plate in the absence of phage, it grew and sporulated heavily. In the presence of the phage it gave a heavy mycelial growth showing some rosette type growth but no dry aerial sporulation.

Other species of *Streptomyces* which produced streptomycin were not affected by the phage. These cultures were kindly sent by Dr. K. B. Raper and Dr. R. G. Benedict from the Northern Regional Research Laboratories at Peoria, Illinois. The cultures were *Streptomyces griseocarneus* NRRL No. *B-1068* (Hydroxystreptomycin producer), *S. bikiniensis* NRRL No. *B-1049* (streptomycin), *Streptomyces* sp. Lilly No. *4* (streptomycin) and *Streptomyces* sp. NRRL No. *B-1350* which is Abbott No. *Na232-M1* (Hydroxystreptomycin).

Several other Actinomycetes which were tested and found to be resistant to the action of the phage were *S. carneus* ATCC-*6847*, *Streptomyces* sp. Wis. *35* (antimycin), *Streptomyces scabies* ATCC-*10246*, *S. flavus* ATCC-*3369*, *S. albus* ATCC-*618* and *3004*, *S. hygroscopicus* CBS and ATCC-*10976*, *S. roseochromogenus* ATCC-*3347*, *S. venezuelae* NRRL No. *B-902* (chloromycetin), *S. fradiae*, Waksman's *3535* (neomycin), *S. ramosus* NRRL No. *B-2334* (terramycin), *S. aureofaciens* NRRL No. *B-2209* (aureomycin), *S. olivochromogenus* ATCC-*3336* and NRRL No. *B-1341*, *S. lavendulae* (streptothricin), *S. sulphureus* ATCC-*3007*, *Streptomyces* sp. Gottlieb (endomycin), *Streptomyces* sp. Wis. *A-158* (helixin), *S. olivaceus* ATCC-*3335* and NRRL No. *B-1224*, *Nocaria corallina* ATCC-*999*, *Nocardia asteroides* JS-*10*, *Nocardia farcinica* ATCC-*3318*, *Actinomyces bovis*, *Micromonospora* sp. ATCC-*10026*, *Mycobacterium tuberculosis*, *M. phlei*, *M. smegmatis*.

On the other hand not only *S. griseus* but also *S. olivaceus* NRRL No. *B-1125*, *S. griseolus* ATCC-*3325*, and *S. viridis* ATCC-*3372* were susceptible to the action of the *S. griseus* phage. With these cultures and using pure cultures of phage, typical plaques of phage were produced as well as the lysis of the spores and mycelium with the consequent multiplication of the phage particles.

These facts clearly demonstrated the polyvalent nature of the *S. griseus* phage which was able to attack and lyse four or more different species of *Streptomyces*.

Two main and strikingly different types of resistant colonies were found among the surviving population of *Streptomyces olivaceus* NRRL No. *B-1125* after the phage attack; one was a new type, sulfur-yellow in color, and the other olive-colored, like the parent culture. The growth characteristics in streaked plates noted after five days' growth and thereafter of these two colony types in agar media A and B at 25° C and at 28° C, can be described as follows:

1. *Sulfur-yellow Colonies:* The most numerous ones constituting about 60 to 90 per cent of the total of phage-resistant colonies. Surface growth abundant, little spreading, very finely corrugated or folded throughout, giving a spongy to rosette-like appearance. Top and the reverse are of the same sulfur-yellow coloration. A faint yellow-soluble pigment is produced by these colony cultures. In medium B the growth and color are more intense than in Medium A. The growth is scraped off easily and in distilled water gives a granular suspension; under the microscope the mycelium is sulfur-yellow; it contains practically no spores or very few. The growth of these colonies, in general, resembles very much that of the various species of the genus *Mycobacterium*.

2. *Regular, Olive Color, Colonies:* They constitute about 10 to 40 per cent. These colonies are of the same appearance as the parent culture which never was exposed to phage. The surface growth is abundant, spreading, developing deeper into the medium than the sulfur-yellow colonies; smooth appearance with a few folds especially at the edges of growth; very heavy aerial sporulation of a light to dark olive color of a powdery appearance; reverse of colony from a dark olive color to an almost black. A faint olive colored soluble pigment diffuses out into the agar medium.

It was of interest to note that *S. griseus* and *S. olivaceus*, which were two of the best microorganisms used in the commercial production of vitamin B_{12}, were both affected by the same phage. Pure cultures of phage derived from single plaques developed in *S. griseus* cultures were able to attack *S. griseus* and *S. olivaceus*, and single plaques from *S. olivaceus* were able to destroy *S. griseus* as well as *S. olivaceus*. Single spore isolations of *S. olivaceus* were made with the aid of a micromanipulator and the cultures after de-

velopment were tested and found to be as sensitive to the action of the phage as their parent culture.

DEVELOPMENT OF IMMUNE AND RESISTANT COLONIES OF S. GRISEUS

The most important measure in phage control is the development of an immune or a highly resistant strain of *Streptomyces griseus* to the attack of the virus. The different cultures of *S. griseus* show various degrees of resistance to a given phage.

SINGLE COLONY ISOLATION: Resistant colonies of *S. griseus* were easily obtained from agar or liquid media cultures grown in the presence of the phage. The addition of 100 mcg of streptomycin per ml of medium helped in the inhibition of *S. griseus* strains which were low or non-streptomycin producers. The presence of streptomycin did not interfere at all with phage growth. In over 200 single colony cultures tested, it was shown that the phage grew and multiplied equally well, and sometime better, in the presence of 100 mcg of added streptomycin per ml of medium as compared with medium without added streptomycin.

A. From Solid Media. Medium A or B, or any other suitable one, containing the phage (one million to a billion phage particles per ml) and a heavy spore suspension of *S. griseus,* sensitive to phage, was poured out and incubated at 25–28° C for 1–2 weeks after which time colonies were isolated. Agar plates containing the phage were streaked on the surface with the sensitive strain of *S. griseus,* and later on the resistant colonies which grew were isolated.

B. From Agitated Cultures. The production of phage resistant strains of *S. griseus* can be accomplished using medium A in shake flasks. If a defoamer is desired, lard oil can be used at 0.1 ml per 100 ml of medium. The shake flasks were sterilized at 120° C and 15 lbs pressure for 20 minutes and each flask (liter capacity) contained 200 ml of medium. The shaker was set at about 92 reciprocating strokes per minute. Each flask was inoculated at the same time with a potent phage filtrate and with a heavy spore suspension of a currently used strain of *S. griseus* sensitive to phage. One hundred micrograms of streptomycin per ml. of medium was added in this first step. The flasks were placed on the shaker at 25° C

for 24–48 hours. A pelleted type of growth resulted. These clearly conspicuous (mycelial growth) pellets were the phage-resistant colonies of *S. griseus*, most of which originated from a single spore. It is important to point out that agitated cultures should not be run over 48 hours because the mycelium will start fragmenting and the culture will deteriorate, making it undesirable for single colony isolation.

The pellets from the flasks were then plated out in agar medium A containing 100 mcg of streptomycin per ml to grow and sporulate, for the single colony isolation. About 0.1 to 1 ml of broth from infected flasks containing the phage and the pellets was used to inoculate each 100 ml of agar medium at 42–45° C, prior to pouring the plates. About 10–15 ml were used per petri plate. After the agar had solidified the plates were incubated at 25–28° C for 1–2 weeks before colony selection was made.

Using the above procedure it was possible to isolate several colonies of *S. griseus* per plate, showing varying degrees of resistance and even immunity to phage. Well developed healthy and sporulated colonies were selected for isolation to agar slants of medium A or B. The agar slants contained the phage and 50 mcg of streptomycin per ml. The slants were grown for 7–10 days at 25–28° C and then tested in medium C in shake flasks for streptomycin and B_{12} producing abilities. The best cultures selected did not require the incorporation of any more phage in the medium in successive transfers.

MASS COLONY RESISTANCE: The mass colony resistance from submerged cultures or from agar media usually produced lower yields. Several experiments were run by developing good mycelial growth in shake flasks within 24–48 hours from heavily inoculated cultures with spores of a phage-sensitive strain of *S. griseus*, to which phage was added at the same time. This submerged inoculum, from 5 to 20 per cent by volume, was used to inoculate fresh medium. As a result the streptomycin yields were low. When this phage-mass-resistant mycelium was used to inoculate agar media A or B, or any other media, good mycelial growth was obtained but aerial (buffish) sporulation was very scanty. For details see plate 3 in FIG. 2 and tube 4 in FIG. 3. This was in contrast to the phage-sensitive strain (never exposed to phage) which always

FIG. 2. Phage action upon *S. griseus U-203* cultures 3 weeks old at 25° C. Plate 1: Control plate seeded with *S. griseus U-203* without phage; it shows heavy growth and sporulation. Plates 2 and 4: Seeded, as plate 1, with the same amount of spores of *S. griseus U-203* but adding phage (100 million particles per ml). Note the different types of resistant colonies which developed. Plate 3: Shows three streaks of growth. Single layer phage plate was inoculated with the 3rd generation through submerged liquid culture of a phage mass resistant culture. It shows poor sporulation and numerous yeasty (rosette) colony types throughout each streak.

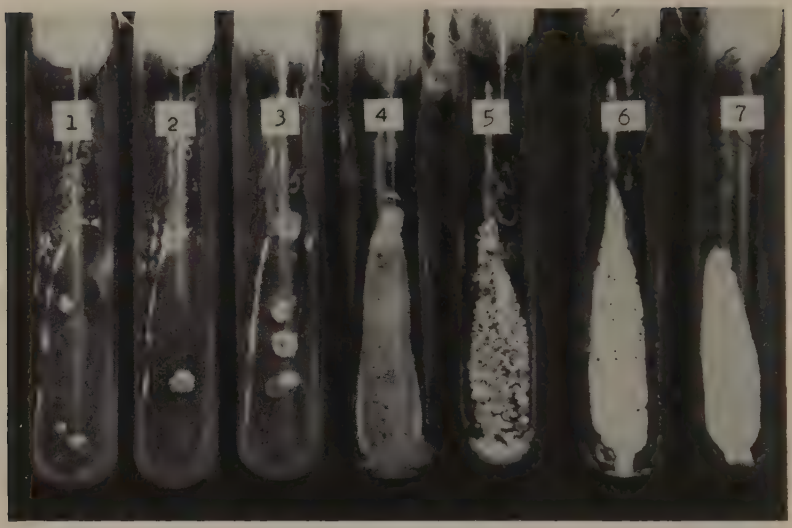

FIG. 3. Phage action upon *S. griseus U-203* cultures 10 days old at 25° C. Various colony types. All the agar slants contained the phage, 100 million phage particles per ml. Tubes 1, 2, and 3: These agar slants which contained the phage were heavily seeded on the surface with phage sensitive culture of *S. griseus U-203*. The various colony types which are shown growing here are the phage-resistant ones. Tube No. 2 contains a typical granular type colony. Tube No. 3 shows a yeasty (rosette) type colony of a nice size toward the bottom of the slant. Tube 4: Inoculated with the mycelium of a submerged third generation of a mass colony (mycelium) resistant culture. Note the poor sporulation, the phage plaques, the varied types of growth and a large yeasty colony type growth toward the center of the slant. Tube 5: Inoculated with spores from a resistant colony obtained from an agar plate. Note the rise of various colony types and the general phage action. Tube 6: Contains a nice resistant colony culture which still is attacked by the phage (fourth generation) as is shown by the number of phage plaques. Tube 7: Shows a heavy sporulation and phage-immune culture of *S. griseus*.

sporulated heavily on agar medium even after passing 12 transfers (every 24 hours) through submerged mycelial growth. In both cases the streptomycin yields were greatly lowered by the end of the twelfth submerged transfer.

S. GRISEUS COLONY TYPES RESULTING AFTER PHAGE ATTACK

FERMENTATION: The action of phage on a sensitive culture of *S. griseus* brought about a variety of colony types which were

strikingly different in morphological, cultural, and biochemical characteristics. These varying types among the surviving population were comparable to those mutants produced by the action of ultraviolet light, X-rays, and nitrogen mustard gas. Therefore, we are making use of this biological principle in our mutation or selection program.

In the mutation work of *S. griseus* for streptomycin and B_{12} production, phage can be used alone, or in combination with one or more of the other agents for selection and mutation such as streptomycin, ultraviolet light irradiation, X-rays, nitrogen mustard gas, or any other mutagenic agent.

When agar dilution plates were made of spores of a phage-sensitive *S. griseus* culture plus phage, with or without streptomycin, five main colony types were developed within one to three weeks, as follows:

1. *Pin Point Type Colonies:* Pin point or very small colonies on the surface or submerged in the medium, mostly without sporulation and hard; some very soft; with little or no color.
2. *Poor Sporulation Type Colonies:* Flat, thin, strongly adhered to the agar, usually of a fairly large size. Often dark in color, with or without dark soluble pigment; some colonies almost colorless.
3. *Regular or Normal Type Colonies:* Typical *S. griseus* well-developed colonies, powdery, of buff to olive greenish color aerial sporulation. This is the type preferred for isolation.

These colonies of the regular or normal type showed varying degrees of resistance as described below:

Nice growth and sporulation was seen after plates were incubated from one to three weeks. By looking at them closely with the naked eye one can easily see their sporulation types. Most of the colonies appeared to have chewed-up or moth-eaten areas in various patterns, on the top or at their edges.

The surface of a single colony may show a few or several large and small areas with varying degrees of sporulation from heavy to almost naked. Some details described here can be seen in FIG. 2, plates 2 and 4. When sub-cultures from the spores of these colonies (see FIG. 3, tubes 5 and 6) were made to agar slants, they usually had from 1 to 100 phage plaques and sometimes more, so

as to be almost completely without sporulation, just mycelial growth. The phage-resistant cultures of poor sporulation or with many plaques were usually poor streptomycin producers, and the results obtained were very inconsistent. Some of these cultures, which were fairly good producers of streptomycin but still carried the phage, were a menace because new strains of phage which attacked and destroyed this culture were produced during fermentation.

Immune Cultures: Among the resistant colonies, there were a few with a nice growth, exhibiting smooth and complete sporulation without any moth-eaten areas. These colonies, upon transfer, usually produced perfect cultures without plaques and without moth-eaten areas (FIG. 3, tube 7). Some of these cultures were immune to the phage action; they did not produce any plaques even when large amounts of phage were added and the phage failed to multiply in these cultures growing in agar or in liquid cultures. After 20 generations, they were still phage-immune, as shown by various tests performed. Furthermore, no phage was recovered after complete autolysis of the mycelium from submerged cultures. This was the ideal type of culture. The cultures chosen for commercial

TABLE I

RESULTS OF 130 REGULAR TYPE CULTURES TESTED IN SHAKE FLASKS. MEDIUM C[1]

Number of cultures	Range of streptomycin production mcg per ml
1	None
42	200–600
4	600–800
25	800–1000
40	1000–1400
11	1400–1600
5	1600–1800
2	1900–2200

[1] *Shake Flask Medium C:*

Distilled or tap water	1 liter
Soybean meal, 4S (Staley's)	20 grams
Corn steep liquor	10 grams
Dextrose (or cerelose)	10 grams
Dextrin	10 grams
$MgSO_4 \cdot 7H_2O$	3 grams
Cobalt (as chloride, sulfate or nitrate)	0.25 ppm

pH adjusted to 7.5 with NaOH before sterilization (after sterilization pH 6.5)
Volume 200 ml per liter flask
Lard Oil 0.2 ml per flask
Sterilization 20 minutes at 120° C, and at 15 lbs pressure.

TABLE II

RESULTS OF 50 GRANULAR TYPE CULTURES TESTED IN SHAKE FLASKS. MEDIUM C

Number of cultures	Range of streptomycin production mcg per ml	Range of B_{12} production mcg per liter
6	None	147–710
17	10–25	119–295
16	26–50	109–228
4	55–80	176–218
2	200–300	96–200
2	400–500	109–130
3	600–700	141–320
Control (parent culture)	1100	400

production were of this immune type. The final choice was made from those cultures of this group which showed consistently high production of streptomycin and B_{12}.

TABLE I shows the results in shake flask fermentations of 130 cultures of the regular type colonies described above.

The control, parent culture *S. griseus U-203,* produced from 1000 to 1400 mcg of streptomycin per ml of medium C; and about 400 mcg per liter of B_{12}. The pH of the broth rose within 1 to 7 days from 6.5 to 8.2–8.6. All the cultures grew very well, and the pH changes as well as the B_{12} yields were very similar to the control culture. The greatest difference was in streptomycin production, in which about 12 cultures out of 130 tested in this experiment proved to be superior to the parent culture *U-203* (phage sensitive). All the regular type cultures seem to be more proteolytic than the other colony types tested here. These excellent results were obtained by treating culture *S. griseus U-203* with phage No. 1. But treatment of another culture, *S. griseus U-203-09,* in the same way but with another phage, isolated No. 39, failed to produce a single superior streptomycin producing strain among 400 strains so tested.

4. *Granular Type Colonies:* Colonies of granular appearance and consistency vary from very small to large and are much raised—rugose. These colonies are usually of a slight pink coloration; appearing from about 0.1 to 1% of the total of colonies. They are of a hard consistency and make a granular suspension (see FIG. 3, tube 2).

In TABLE II the shake flask fermentation results obtained with 50 granular type cultures are summarized.

The type growth and the pH behavior of the granular cultures in shake flasks were very much the same as the parent control cultures but all the granular cultures, without exception, gave inferior streptomycin yields. All the cultures produced B_{12}, but less than the parent culture, with only two exceptions. Culture *354* produced 620 mcg and culture *355* produced *710* mcg of B_{12} per liter of broth; however, these two cultures did not produce any streptomycin.

5. *Yeasty Type Colonies—Rosette:* Yeast-like type colonies appear wet, like a culture of yeast or bacteria, very soft; some are mucilaginous. They appear with a frequency of about 0.1 to 5%. They may appear as flat or as wrinkled or in donut and rosette shapes (see FIG. 2, plate 3 and FIG. 3, tubes 3 and 4). The color varies from dirty white to slight yellowish. These colonies are scraped off easily and all of them make a nice and homogeneous cloudy cell suspension. Microscopically they have regular size spores and some cultures have a few very large spores from 1 to 3 microns and even more in diameter resembling sporangia of some Phycomycetes; the mycelium usually short, in fragments, very often continuous and worm-like or with little ramification, or bearing spores singly or in chains. Most of these strains seem to be lysogenic. Most of these cultures produce a dark, sometimes almost black, soluble pigment in agar medium and liquid medium. A few produce no soluble pigment. In shake flasks they grow and sporulate (submerged) like the parent culture; but mycelium often in shorter threads; some cultures had very thin mycelium, some of larger dimensions, some straight, some very wavy and coarse, some long and very branched, some very short, when compared with parent culture (never treated with phage).

A total of 50 different cultures of these types were tested in shake flasks for growth, streptomycin, and B_{12} production in *medium C* and the results are tabulated in TABLE III.

All the above 50 cultures produced vitamin B_{12}, and produced it at an earlier date than the control parent culture. The pH increased from 6.5 to 8.0 within 1 to 8 days more slowly than in the control culture. As a general rule, in shake flask fermentation these cultures grew more slowly than the control and the growth of most of them was never as profuse.

With reference to streptomycin production, out of the 50 cultures only nine produced some streptomycin. But of these nine

TABLE III

RESULTS OF 50 YEASTY TYPE CULTURES TESTED IN SHAKE FLASKS. MEDIUM C

Number of cultures	Range of B_{12} production mcg per liter
28	301–400
9	401–500
5	501–600
4	601–700
3	701–800
1	901–1000
Control (parent culture)	400

cultures, eight produced only from 15–30 mcg of streptomycin per ml of broth and from 300–400 mcg of B_{12} per liter of broth. The ninth produced 270 mcg of streptomycin and 700 mcg of B_{12} per liter of broth. The other 41 cultures produced no streptomycin, all the high B_{12} producers being among them.

PRESERVATION OF HIGHLY PRODUCTIVE CULTURES OF S. GRISEUS

Once a culture of *S. griseus* has proved to be an excellent streptomycin producer, enough spore inoculum should be preserved. It should be stated here that a good culture should not be passed through many generations before commercial production of streptomycin, because many of the cultures tend to reduce their streptomycin productivity. The lyophilization of the cultures is the best insurance of maintaining a highly productive culture. Therefore enough ampules of desirable size are lyophilized in order to have enough initial inoculum to be used periodically over a period of years. At present, we have three different cultures of *S. griseus* which were lyophilized 8 years ago, and have maintained their biochemical and cultural characteristics identically with the parent cultures of 8 years ago. The lyophilization procedure used here for nearly ten years was described by Wickerham and Andreasen (**16**) and by Carvajal (**4**) using Difco dehydrated skimmed milk. For streptomycin production of lyophilized culture is opened periodically, *e.g.*, each month, and Fernbach's containing agar medium B plus 100 mcg of streptomycin per ml are surface inoculated (2 ml spore suspension per flask) and grown at 25° C for 7–10 days, then used, or stored at 3–5° C until used. The spores of each Fern-

bach are used in suspension to inoculate the seed tanks directly (one Fernbach for each seed tank of 300–500 gallons). The young vegetative inoculum from the seed tanks is used to inoculate the fermenters for the production of streptomycin.

Complementary to the lyophilization of the cultures, *S. griseus* is also kept in soil cultures, cultures under mineral oil, and the regular agar slant cultures (for slants, large tubes 6 inches high and 1 inch in diameter are preferred).

For the soil cultures, spores straight from agar cultures are incorporated into fairly dry garden soil which has been previously sterilized in the autoclave. These soil cultures are immediately stored at 3–5° C. This method has given better results than the usual method (11) of inoculating the soil with a spore suspension and permitting the organism to grow and reproduce for one week or so prior to storage at 3–5° C.

PRESERVATION OF PHAGE

1. *Stock Collection of Phage Filtrates:* It is very important to keep the broth Seitz filtrate of every infected tank. By this means one can detect new strains of phage which normally are produced, and thus be protected against them. These filtrates can be used singly, or in composite samples, in the development and selection of resistant and immune strains of *S. griseus* and in the selection of superior streptomycin and B_{12} producers.

2. *Lyophil Preservation of the S. griseus Phage:* The drying technique used here is essentially the same as that described in 1942 by Bickerham and Andreasen (16) for yeast. The writer used two different steps in the lyophilization of the phage:

A. The suspension of the phage particles in Difco skimmed milk, blood serum or any other protein rich medium.
B. The suspension of the phage as in A, plus fresh spores of a phage-sensitive strain of *S. griseus*.

These suspensions are immediately lyophilized. Strains of phage lyophilized in skimmed milk as in A above and kept at room temperatures for a period of two months have been found to retain their initial physiological and biochemical characteristics.

SUMMARY

Effective measures for the control and eradication of the phage contamination depend upon strict sanitary precautions throughout the laboratories and fermentation areas and mainly upon the development of cultures immune to the action of the phage.

The end result of the virus infection in the fermentation tanks is usually a characteristic loss of viscosity, diminution or absence of mycelial growth, little or no streptomycin, the presence of a dark brown soluble pigment and the presence of the phage.

The action of phage in a sensitive culture of *S. griseus* brings about a variety of colony types which are strikingly different in morphological, cultural, and biochemical characteristics. These varying types among the surviving population are comparable to mutants produced by the action of ultraviolet light, X-rays, and nitrogen mustard gas. With the development of phage-immune strains of *S. griseus,* not only was the phage eliminated but also the streptomycin yields were significantly increased.

Of the various Actinomycetes cultures tested, strains of *Streptomyces olivaceus, S. griseolus,* and *S. viridis* were sensitive to the phage as well as *S. griseus.* Therefore, the *S. griseus* phage is of a polyvalent nature, being able to attack and lyse four or more different species of Streptomyces.

The mechanism of action which determines the lysis of the spores and mycelium of the fungus *S. griseus* and the formation of plaques is dependent on the availability of free water in the medium which will help in the diffusion of the phage and in the penetration and lysis of the cells by the phage.[2]

The writer wishes to acknowledge appreciation for the assistance of Mr. Ralph Hill, Mrs. Anna Mae Cottingham, Mrs. Mary Spargo, and Mrs. Verna Schulenberg, to Mr. James Burns who performed all the streptomycin and vitamin B_{12} assays, and to Mr. Gabor B. Levy for the photographic work.

[2] After the manuscript for the present paper had been completed, a publication by J. Bronfenbrenner appeared in Science **116**: 517, November 14, 1952. Some of the observations made by Dr. Bronfenbrenner may be explained by the same phenomena.

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SIZE IN RELATION TO THE RATE OF MIGRATION IN THE SLIME MOLD DICTYOSTELIUM DISCOIDEUM [1]

JOHN TYLER BONNER, PAUL G. KOONTZ JR., AND DAVID PATON

(WITH 2 FIGURES)

It is characteristic of the Acrasiales or amoeboid slime molds that a group of uninucleate amoebae aggregate, but in only one species described thus far, *Dictyostelium discoideum* Raper, does this cell-aggregate or pseudoplasmodium migrate for a period of time before the final fruiting or culmination. The migrating pseudoplasmodium has a sausage or cartridge shape and moves slowly over the substratum secreting a slime sheath that is deposited as a track posteriorly. It has been studied by a number of investigators [2] and found to have numerous interesting structural and physiological properties, although many aspects of its behavior remain unexplained, including the mechanism of its motion. In order to collect more information concerning this interesting stage of development, a study has been made of the rates of movement and it was found that similar to the rates of culmination (3) the larger the pseudoplasmodium, the greater the speed of migration. As will be shown, this lends evidence to the hypothesis that the propelling force for movement is internal and depends on the total mass of cells rather than a superficial cell layer.

METHODS

The slime mold was grown in a two membered culture with *Escherichia coli* on the same nutrient medium used in previous studies (2). Prior to migration, when the cells had just assembled

[1] This work was carried out with the help of a grant from the American Cancer Society and with funds of the Eugene Higgins Trust allocated to Princeton University.

[2] See Raper (6) for references to the work of Raper, Gregg and Bonner. Also there is some more recent work of Slifkin and Bonner (7).

into pseudoplasmodia, certain of these were transferred with a steel spatula to the surface of a non-nutrient, 2% agar medium. As has been shown by Slifkin and Bonner (7) this medium favors prolonged migration, thereby facilitating prolonged observations on the same pseudoplasmodium. During both the growth and the subsequent migration stages the petri dishes were kept at 20.5 ± 0.5° C. (in the dark) and during the migration period the dishes were kept within a closed moist chamber to prevent desiccation.

As soon as migration started a camera lucida drawing was made of the pseudoplasmodium, and this was repeated at approximately 12- or 24-hour intervals. The distance travelled was traced (by following the slime track) with india ink on the bottom of the petri dish. This was later measured by placing the petri dish bottom in a lantern slide projector and tracing the projected track on the screen with a map mileage indicator and making the necessary unit conversions. The volume of the pseudoplasmodia was estimated by the segment method given in Bonner and Slifkin (4). Since

TABLE I

SHOWING THE RELATION BETWEEN THE VOLUME OF MIGRATING PSEUDOPLASMODIA AND THEIR RATE

(The volumes are a calculated mean of the volume before and after a given period of migration for which the rate has been determined.)

Pseudo-plasmodium number		Volume in mm.³	Rate in mm./hr.	Pseudo-plasmodium number		Volume in mm.³	Rate in mm./hr.
1	1	.0438	2.00	5	1	.0168	.92
	2	.0400	1.75		2	.0153	.59
	3	.0350	1.25		3	.0129	.33
					4	.0103	.25
2	1	.0337	1.25				
	2	.0238	1.04	6	1	.0236	1.63
	3	.0177	.83		2	.0209	1.14
	4	.0163	.79		3	.0195	.80
					4	.153	.70
3	1	.0259	1.61		5	.0126	.68
	2	.0224	1.16				
	3	.0155	1.00	7	1	.0414	1.26
	4	.0118	.76		2	.0399	1.17
	5	.0101	.72		3	.0315	.47
	6	.0064	.61		4	.0247	.48
	7	.0048	.53				
				8	1	.0437	1.33
4	1	.0412	1.50		2	.0363	1.07
	2	.0396	1.33		3	.0255	1.01
	3	.0337	.95		4	.0217	.63
	4	.0245	.91				

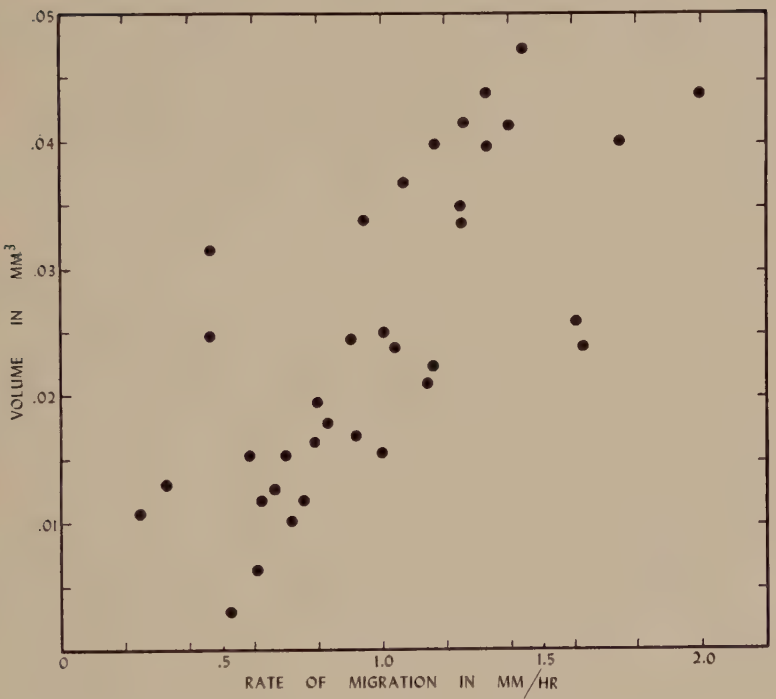

FIG. 1. Graph showing the relation of the volume of migrating pseudoplasmodia (ordinate) to their rate of movement (abscissa).

the rate is given by distance between two observation points (camera lucida drawings) in a given time, the volume equivalent to this rate was estimated by obtaining the mean volume of the two drawings.

RESULTS AND DISCUSSION

The results for eight different migrating pseudoplasmodia are given in TABLE I, and if each average volume is plotted against the rate, it is obvious that the larger the volume, the greater the rate (FIG. 1). This fact is even more clearly seen in an individual pseudoplasmodium, for as migration proceeds the pseudoplasmodium becomes smaller, and its speed is correspondingly slower (see TABLE I and FIG. 2). The reason for the decrease in size during migration is probably two-fold: some of the cells straggle and are lost in the slime track, and also the pseudoplasmodia expend energy without taking in food.

These results parallel previous work on culmination (Bonner and Eldredge, 3), where again the larger the pseudoplasmodium the greater the rate of upward rise into the air. As was pointed out then, the results on culmination, and now we may include the ones given here on migration, fit in with the hypothesis involving the

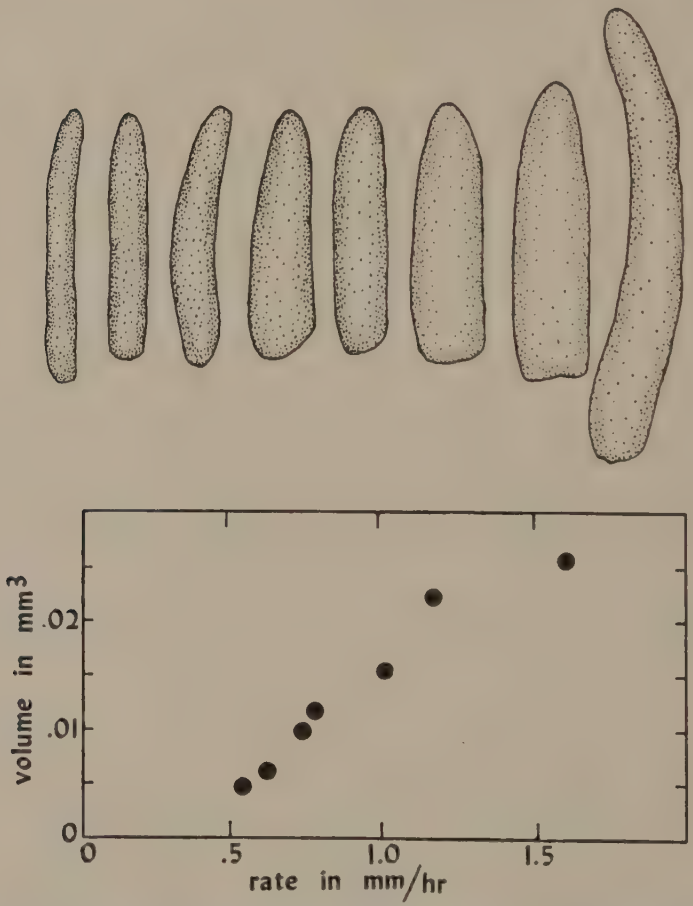

FIG. 2. *Above.* A series of camera lucida drawings of a single pseudoplasmodium (No. 3 in TABLE 1) showing, from right to left, the gradual decrease in size upon migration. *Below.* A graph of the same pseudoplasmodium in which the ordinate is the volume and the abscissa the rate of movement. (The volume values are each a mean between two of the camera lucida drawings above, corresponding to a particular rate measurement.)

principle of similitude originally proposed by Tyler (8) for a somewhat similar situation in sea urchin embryos.

According to the principle of similitude, in similarly shaped bodies of different size, the volume of surface ratio will increase with size, for volume increases as the cube of the linear dimensions, but the surface as the square. Since large migrating pseudoplasmodia move faster, the locus of the movement force is likely to be dependent on the volume or mass rather than the surface. On the other hand the resistance to movement or friction could be a surface phenomenon. This hypothesis assumes that the power of movement per unit mass of cells is constant for different size pseudoplasmodia, and Gregg (5) has shown that this assumption is supported by the fact that the oxygen consumption per unit nitrogen of tissue is constant irrespective of size.

There has been much speculation concerning the nature of this migration movement and relatively few facts. It is known that the internal amoebae are actively pseudopodial and that the individual amoebae may, to some degree, travel at different rates (Bonner, 1). We may now postulate from the evidence presented here, that all the amoebae are actively involved in the forward progression and somehow gain traction from both the slime sheath they secrete and from one another below the surface of the cell mask.[3] The idea that the forward progression is achieved solely by the outside, superficial layer of cells is not supported by the present evidence.

SUMMARY

In the amoeboid slime mold *Dictyostelium discoideum,* separate amoebae aggregate to form sausage-shaped migrating pseudoplasmodia. The rates of movement of these pseudoplasmodia were found to be proportional to their size. This was true even of indi-

[3] There is no objection to the idea that an internal amoeba might obtain traction from his neighbors provided there is an anchor (the slime sheath) somewhere in the system. An amoeba moves by keeping its sides stiff, in a gelled condition, while the internal fluid protoplasm spouts forward at the anterior tip. If the lateral stiff portions of each amoeba stick to one another they will automatically have their traction. Also it is conceivable that each amoeba gives off slime which has some rigidity for traction and is ultimately deposited inside the slime track.

vidual pseudoplasmodia, for the longer one migrated the smaller it became and its rate decreased correspondingly. An hypothesis was presented, based on the principle of similitude, to the effect that migration movement was dependent on the total mass of amoebae, and not upon a superficial layer of specialized locomotory amoebae.

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DETECTION OF HISTOPLASMA CAPSULATUM AND OTHER FUNGUS SPORES IN THE ENVIRONMENT BY MEANS OF THE MEMBRANE FILTER

Morris A. Gordon and Horace B. Cupp, Jr.

(with 2 figures)

The quantitative determination of fungi in the physical environment usually is undertaken by means of cultural procedures, most commonly by exposure of suitable media in Petri dishes with subsequent counting and identification of colonies. For those organisms possessing diagnostic microscopic structures, more rapid detection may be accomplished by the simpler method of examining petrolatum-coated gravity slides or similar devices (**6**). Among the fungi particularly suited to direct examination methods is the human pathogen, *Histoplasma capsulatum*. At the time the present studies were undertaken, this organism had not been found to occur naturally in air or water, but had been recovered from soil on several occasions (**1**, **3**, **5**). In all of these successful attempts the technique employed was essentially that of Stewart and Meyer (**11**), involving inoculation of a soil suspension intraperitoneally into mice and subsequent recovery of the fungus by culture of various tissues. Emmons was able also to demonstrate directly, in some of his soil suspensions, characteristic tuberculate spores of *H. capsulatum*. Grayston, Loosli, and Alexander (**5**), in obtaining the fungus repeatedly from organic debris in a silo to which human infection had been traced, gave strong support to the popular concept that histoplasmosis is a dust-borne infection. It appears likely, also, that the etiologic agent may be ingested with food or water. In order to aid in the further exploration of these possibilities, and to find a rapid and efficient method for the detection in the environment of fungi in general, the use of the Goetz membrane has been investigated.

The membrane filter, which permits rapid passage of large volumes of air and water while retaining particles the size of bacteria and smaller, is admirably adaptable to the detection of fungus spores in these media. Solid materials such as soils may also be searched with the aid of flotation techniques. The history of the membrane, and accounts of its employment in sanitary bacteriology, are provided in recent papers by Paccagnella (**10**) and Clark *et al.* (**2**). These workers cultured the exposed membranes on absorbent pads saturated with selective and differential media for the isolation of bacterial colonies. Fungi also may be cultured in this manner, but more rapid detection is made possible by direct examination of stained membranes for characteristic spores.

For the latter purpose there has been devised a staining procedure which permits excellent differentiation of fungus structures from other debris as well as from the homogeneous background of the filter disc. Advantages of this filtration and staining procedure over previous methods, direct or cultural, include the following:

1. More rapid detection of fungi. Following filtration, the membrane may be processed and examined thoroughly within an hour.
2. Large sample volume, limited only by the amount of debris in the material being filtered.
3. Efficiency. *All* of the spores in the sample remain on filter discs of suitable porosity.
4. Simplicity of apparatus. Culture media and incubators are not needed.
5. The method is applicable equally to the examination of gases, liquids, and solids.
6. As contrasted with cultural methods, there is no dependence upon the ability of the fungus to outgrow other organisms or to overcome inhibition.
7. It is especially suitable for the rapid detection of gross contamination of the environment.

The chief limitation of this method is the necessity for identifying fungi solely on the basis of microscopic structure. In the case of *H. capsulatum*, the spores seem to be sufficiently distinct to minimize this deficiency.

It may be pointed out that the apparatus and techniques to be described are quite suitable for the counting of pollen grains, which take the stain very well. An additional application, omitting the stain, came to our notice in the course of this work. Legler (9) has employed the membrane filter for counting *Ascaris* eggs in sewage effluent and feces.

APPARATUS

The first filter membranes employed in these experiments were prepared in Dr. Goetz' laboratories at the California Institute of Technology. Subsequent discs ("Millipore Filters, Type HA" [1]) were purchased from Lovell Chemical Co.,[1] Watertown, Massachusetts. All were 50 mm in diameter and of unspecified porosity. The hydrosol filtration apparatus (FIG. 1), designed by Dr. Goetz and obtained from the L. R. Burt Co.,[1] Arcadia, California, is essentially identical with that employed by Clark *et al.* (2), consisting of a stainless steel funnel of one liter capacity connected by a bayonet locking nut to a receptacle containing a porous plate. The filter disc is locked on top of the porous plate, through which the filtrate drains into a filter flask.

Suction was obtained by means of either an electric vacuum pump or an aspirator operated on water pressure from a tap. Inserted in the line between the aspirator and the filter flask were a one-liter flask to serve as a water-trap and a side-arm with screw clamp for regulating the pressure. In field operations where water-pressure lines and vacuum pumps are not available, sufficient suction may be obtained by utilization of the exhaust manifold of a motor vehicle.

Although inferior in some respects to the filter membrane for direct examination, certain grades of filter paper, having the advantages of more ready availability and less expense, may also be employed for the retention of fungus spores. Whatman filter papers No. 3 and No. 50 have served successfully when fitted in Seitz filters of 30 ml. or 100 ml. capacity. The filtration speed of the No. 50 disc approximated that of the membrane, while No. 3 was much more rapid. All three serve equally well for cultural

[1] Trade names and commercial sources are mentioned as a means of identifying the products under discussion, and their use is not intended to represent endorsement of the products by the United States Public Health Service.

methods, but the Goetz membrane has the advantage of optical homogeneity for direct microscopic examination. However, the smooth surface of the membrane permits trapped spores to be washed off more readily during the staining process. It is possible also, in employing membranes, to substitute Seitz apparatus of suitable size for the more specialized filter equipment. With such an arrangement a reinforcing disc of blotting paper must be placed

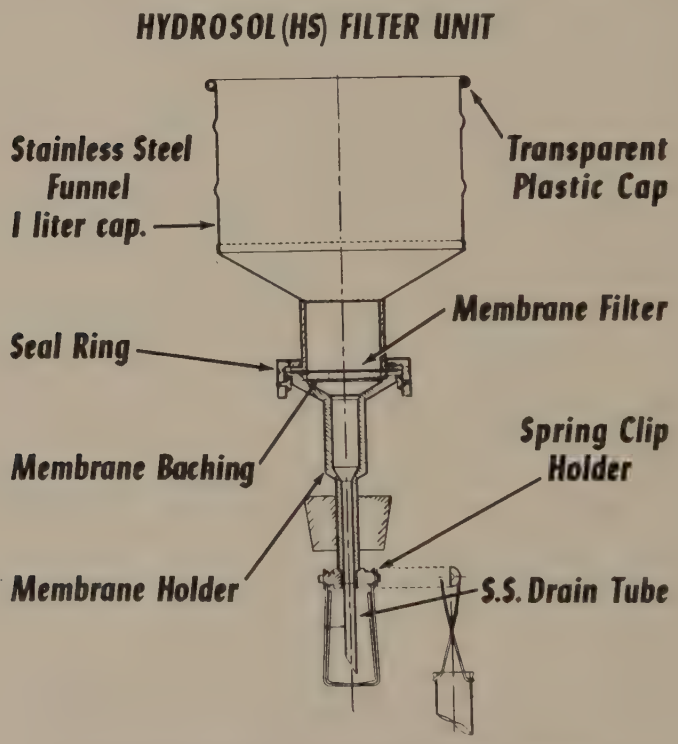

FIG. 1. Line drawing of Goetz hydrosol filtration apparatus.

between the supporting wire grid and the membrane in order to prevent rupture of the latter.

In air-sampling experiments, either a closed Seitz filter (for pressure and vacuum) or a Goetz-designed aerosol filter apparatus was used. The aerosol filter is similar to the hydrosol type, but the funnel of the latter is replaced by a closed top with air inlet, similar to that in the Seitz pressure-vacuum filter.

EXPERIMENTAL HYDROSOL FILTRATION

The filter membrane and several grades of Whatman filter paper of varying retentivity were tested with aqueous suspensions of *H. capsulatum* tuberculate spores for ability to retain these spores and for speed of filtration. In the retention tests, the filter disc was either cultured or examined microscopically, while the filtrate was cultured on both Sabouraud dextrose agar and Sabouraud dextrose broth. It was found that Whatman papers No. 3 and No. 50 and the membrane completely sterilized *H. capsulatum* spore suspensions, while Whatman No. 1 filtered out most of the spores but permitted some to pass through, as was evidenced by growth in the filtrate cultures. The maximum rate of filtration of tap water obtainable with the membrane filter apparatus, using an electric vacuum pump, was about 30 liters per hour. At a comparable pressure, Whatman No. 3 paper supported in a Seitz apparatus, either large or small, permitted a flow of 48 liters per hour. Application of greater pressures in an attempt to increase this rate resulted in tearing of the paper, although reinforcement of the No. 3 with 2 discs of No. 1 permitted a flow of 60 liters per hour without rupture. The filtration speed of the No. 50 paper in a Seitz apparatus approximated that of the No. 3, but the former sometimes permitted leakage around the periphery unless supplied with a rubber or cork gasket.

STAINING OF THE FILTER DISC

The staining procedure used for all filter discs was adapted from the Hotchkiss-McManus (periodic acid-Schiff) method for differentiation of fungus structures in animal tissue (8). The disc is removed with forceps from the filter apparatus and transferred to a small (60 × 15 mm.) clean Petri dish. The following reagents are in turn poured into the dish and discarded after the stated time intervals: periodic acid, 1% aqueous—5 minutes; tap water—3 changes totaling 5 minutes; Schiff reagent—5 minutes; potassium metabisulfite solution (5 ml. of 10% aqueous potassium metabisulfite and 5 ml. of normal hydrochloric acid in 100 ml. of distilled water; mixture to be prepared fresh daily)—3 changes of 5 minutes each; tap water—rinse twice, then apply fresh water for 5 minutes. The moist disc is then transferred to a 2 × 3 inch glass microscope

slide and covered with a 45 × 50 mm. coverslip, care being taken to exclude air bubbles. Water is added by capillarity if necessary. The preparation is examined systematically upon the compound microscope under low power (100 ×) with transmitted light. For critical examination of individual objects, high power (440 ×) may be used without significant loss of definition. During the examination, water is added as necessary to keep the area beneath the coverslip sufficiently moist for preservation of translucency, but care must be taken to avoid an excess of water which will cause the disc to float, thus interfering with sharp definition.

In such a preparation *Histoplasma* spores and other hyaline fungus structures are stained a deep pink or red and are strikingly visible against a very pale pink or colorless background (FIG. 2, a). Dematiaceous spores or hyphae, although not visibly stained, also stand out. Pollen grains take the stain well and details are particularly well defined. Examination of the membrane disc is facilitated by the presence of a ruled grid upon its surface. With the 16 mm. (10 ×) objective the diameter of the microscopic field is almost exactly half that of the grid units, with which most membranes come supplied, so that in surveying the disc under low power a guide line is always present at one edge of the field. When a suspected object is sighted it may be examined more critically with the 4 mm. objective.

Early in the course of this study it was suspected that, on account of the evenness of the membrane surface, some spores might be washed off during the staining process. That such was actually the case was confirmed by saving all reagents (except the Schiff stain) used in the processing, combining them, and later filtering the mixture through a No. 3 disc. Upon staining and examination of the latter, numerous spores were found. Some sort of pre-stain fixative was therefore necessary, which would not, however, interfere with the staining results. Haupt's adhesive (7) was found to suit the purpose. It is applied to the exposed membrane in a thin layer by means of a dropper and must be permitted to coagulate before initiation of the staining. Collodion (Collodion Flexible, U.S.P.) may also be used and has the advantage of rapid evaporation of the solvent, but it is difficult to apply evenly without disturbing the surface of the disc.

The Haupt fixative has been employed routinely also with the No. 50 paper, but is not considered necessary for the No. 3 paper, which has a coarse fibrous composition. As has already been pointed out, these filter papers, on account of their lesser homogeneity, are not so suitable for microscopic examination as the membrane, but may be employed satisfactorily and do permit excellent spore differentiation upon staining.

PERMANENT MOUNTING OF STAINED DISCS

Stained membranes and Whatman papers may be stored dry and subsequently rehydrated for examination, but repeated drying and moistening eventually impairs the sharp differentiation of spore details. For temporary storage a moist chamber may be used, but it is possible to make good permanent preparations by means of a mounting resin, as follows:

1. Allow the disc, with slide and coverglass, to air-dry.
2. Rim the coverglass with an excess of a xylene- or toluene-based mounting resin (HSR has been employed in this work).
3. Immediately apply xylene to the rim at one or more points until the xylene-resin solution has, by capillarity, saturated the entire disc.
4. Allow to harden, rimming with additional resin as needed.

Upon hardening of the mounting medium such a preparation remains transparent (FIG. 2, b), with spores readily discernible microscopically (FIG. 2, c–h). It is sometimes necessary to provide additional xylene or resin in order to cover denuded areas.

PRACTICAL WATER SAMPLING

Limited field trials have been undertaken with samples of drinking water, rain water, and river water from an area in Tennessee where there is a high prevalence of histoplasmin sensitivity among the human population. These samples were collected and filtered by Dr. L. D. Zeidberg of the Tennessee Department of Public Health, Nashville, Tennessee, and the filter discs were mailed to this laboratory for processing. As reported elsewhere (4), a tuberculate spore typical of those of *H. capsulatum* appeared on a membrane through which one liter of river water had been passed.

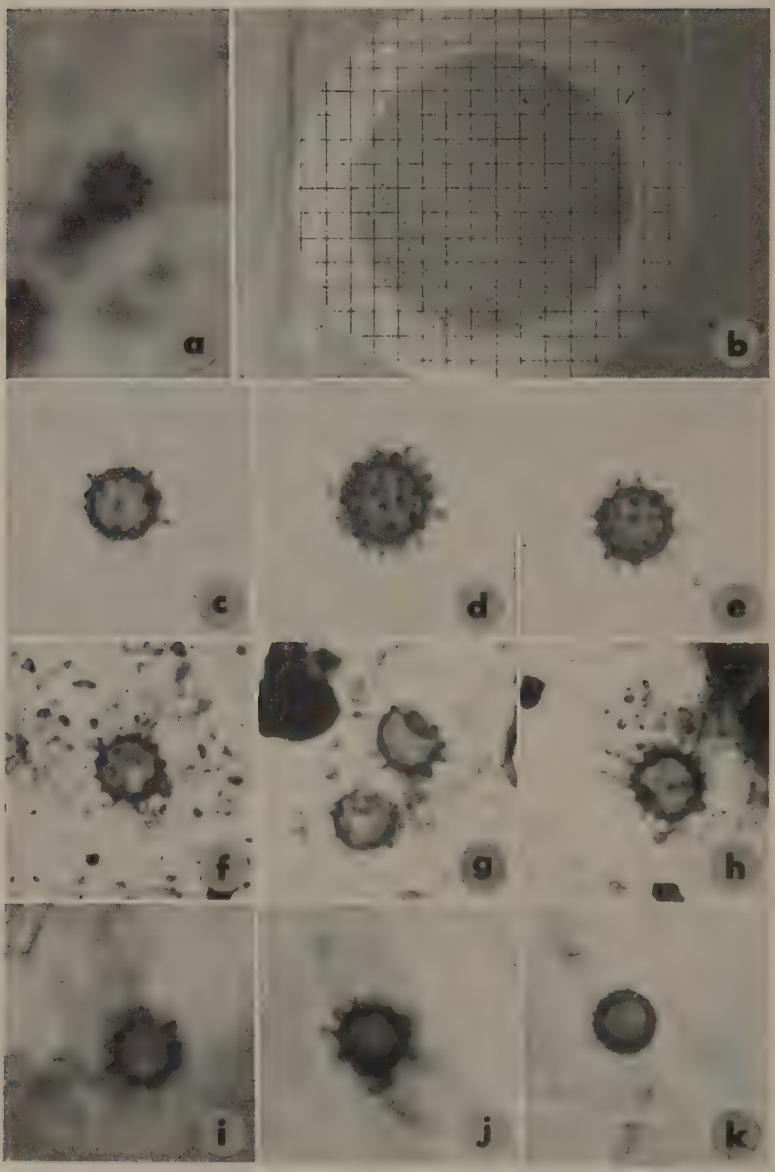

FIG. 2. All material stained by modified periodic acid-Schiff method. a. Tuberculate spore of *H. capsulatum* from soil, on membrane filter. Wet mount, × ca. 600. b. Permanent mount of stained membrane, × 1. c–e. Known *H. capsulatum* spores from artificial hydrosol, on membrane. Permanent mount, × 760. f–h. Spores from soil on membrane. Permanent mount, × 760. i–k. Known *H. capsulatum* spores from artificial aerosol, on Whatman No. 50 filter paper. Wet mount, × 760.

H. capsulatum has not been recovered from the other water sources, but these have yielded many other types of fungus spores, both hyaline and dematiaceous, including those of *Alternaria, Curvularia,* and other species, and large numbers of pollen grains. In practice, one-half to two liters of water have been filtered through each disc, but the usable volume is limited only by the degree of opacity produced by the sediment. In processing a series of samples where distilled water is not available for flushing the filter apparatus between specimens, the apparatus may be rinsed before each run with a small quantity of the material about to be filtered.

In order partially to answer the question of whether *H. capsulatum* spores remain viable in drinking waters for any considerable period of time, thereby constituting a possible health hazard, a series of viability experiments was conducted wherein various water samples, sterilized by Seitz-filtration, were seeded with known concentrations of these spores and sampled periodically by plating for *H. capsulatum* colonies. It was found that a small percentage of the spores remained viable for at least one month in distilled water and 3 weeks in tap water or untreated well water.

RECOVERY FROM SOIL OF *Histoplasma capsulatum* AND *Microsporum gypseum*

Four samples of soil (kindly made available by Dr. L. Ajello), aliquots of which had been found by the mouse-culture technique (1) to contain an infectious stage of *H. capsulatum,* were searched for the tuberculate spores of this fungus by the following procedure: a teaspoonful of soil was triturated in a sterile 25 × 150 mm. test tube with 30 ml. of a sterile 0.85% solution of sodium chloride. The suspension was permitted to settle for from two to several hours and then the entire clear supernatant was filtered through a membrane filter. This was fixed with Haupt's adhesive, stained, and examined. Typical well-stained tuberculate spores (FIG. 2, f–h), 9.5–12 μ in diameter (exclusive of the tubercules), were found in each of the soils, their number ranging from 1 or 2 on the entire disc, in the poorest sample, to an average of at least one per low-power field in the best. The spoonful of soil which had provided the highest count was then re-extracted with an additional 30 ml. of saline and was found again to yield a considerably reduced, but still

appreciable, number of spores. As a result of this and further repetitions, it has become evident that the flotation procedure employed is considerably less than 100 per cent efficient in extracting fungus spores from soil. Emmons (3) previously had recovered *H. capsulatum* spores from soil by means of a similar flotation technique, following which, however, portions of the supernatant were examined directly under the microscope. In the present studies such an examination also was successful with samples rich in spores, but the filtration method has proved to be more efficient. It is not known how many spores or other particles of *H. capsulatum* must be present in the soil inocula in order to infect mice, so that there has been no indication heretofore of the degree of infestation of soils with this organism. The membrane filter method would appear to offer an opportunity for quantitative determination, based on the number of spores present. A comparative evaluation of the two methods for detecting *H. capsulatum* in soil specimens is now under way in this laboratory.

An additional discovery of importance on one of the soil-filtration membranes was the presence of well-stained multicellular conidia typical of the dermatophyte, *Microsporum gypseum*. Resuspension of the soil aliquot which had yielded these spores, followed by streaking of a portion of the supernatant on selective media, resulted in the isolation of this fungus in pure culture. Details of these studies will appear elsewhere.

EXPERIMENTAL RECOVERY OF AIRBORNE SPORES OF *H. capsulatum*

A preliminary experiment on the filtration of artificial fungus aerosols has been concluded. A small quantity of dry *H. capsulatum* spores was mixed with a large volume of talcum powder in a 125 ml. side-arm suction flask equipped with a one-hole rubber stopper. The stopper was fitted with an L-shaped glass intake tube, cotton-plugged at its external orifice and narrowed to a small aperture at the opposite end, which was situated about 3 cm above the surface of the spore-talcum mixture. A length of glass tubing with rubber connecting-pieces led from the side-arm of this flask to the pressure inlet of a large (100 ml.) Seitz filter apparatus, containing a Whatman No. 50 paper disc and rubber gasket and inserted into

a one-liter suction flask. Gentle suction was applied to the side-arm of the large flask for several minutes, until a white coating of powder had begun to appear on the interior wall of the glass connecting-tube. Upon completion of the filtration the filter disc, visibly coated with talc, was moistened, transferred cautiously to a Petri dish, coated with Haupt's adhesive, and stained as above. Numerous well-stained scattered tuberculate spores (FIG. 2, i–k), resembling in all details those recovered from soil specimens and water, were strikingly visible against the unstained talc and filter paper.

FILTRATION OF *Coccidioides immitis* HYDROSOLS

Aqueous suspensions of arthrospores of *Coccidioides immitis* were filtered through membranes and through No. 50 paper fitted into a small Seitz apparatus. The filter discs were stained and the filtrates cultured. In this experiment, filtration through the membrane was more rapid than that through the Whatman No. 50 disc. Each served to retain most of the spores, but neither completely sterilized the suspension, as was shown by growth of *C. immitis* colonies in the filtrate cultures. Arthrospores upon both types of filter disc were well stained, but required tedious search on account of their minute size. Detection of the spores under low power was more difficult on the No. 50 filter, as a result of its lesser optical homogeneity and the trapping of some of the spores in the interstices of the fibers. However, under higher magnification (440 ×) the characteristic structures of *C. immitis* were readily found and easily recognized.

SUMMARY

The membrane filter affords a simple, rapid, and efficient method for detection of fungus spores in hydrosols and aerosols. The exposed membrane may be subjected to a selective stain and examined through the compound microscope by means of transmitted light. Details of this procedure are described.

Whatman filter papers of certain grades and Seitz filters may sometimes serve as acceptable substitutes for the more expensive and less generally available membranes and special membrane filter apparatus.

In field trials of this method, typical tuberculate spores of *Histoplasma capsulatum* have been recovered from several soil samples and from river water. Macroconidia of *Microsporum gypseum* have also been found in soil.

A method is described for preparing stained permanent transparent mounts of the filter discs.

Grateful acknowledgment is made to Mr. Lawrence B. Hall, Communicable Disease Center, Savannah, Ga., for having made available some of the equipment used in this study.

COMMUNICABLE DISEASE CENTER,
PUBLIC HEALTH SERVICE,
FEDERAL SECURITY AGENCY,
ATLANTA, GEORGIA

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EVIDENCE FOR SYNONYMY OF TORULA BERGERI AND PHIALOPHORA JEANSELMEI

A. Trejos

(with 2 figures)

During the course of the study we have made on 30 strains of etiologic agents of chromoblastomycosis, isolated by us in Costa Rica, we have observed, since the middle of 1950, cultures of *Torula bergeri* and pathological material from the case that was diagnosed in Canada by Berger and coworkers.[1] The comparative study of these cultures with those of other agents of chromoblastomycosis and maduromycosis has enabled us to make some observations summarized below.

We received from Dr. Carrión two cultures labeled "*Candida*-like sp. de Berger" with numbers *1074* and *1077*. Strain *1077* corresponds to the one isolated by Berger *et al.* (1) from the last biopsy performed on his patient in 1943. This strain has maintained the same macroscopical appearance that it had when first isolated; that is to say, dry, wrinkled and protruding colonies which do not penetrate the culture media, as described by Berger, Beadry and Gaumond (1) and Berger and Langeron (2). They readily separate in fragments, like powdered charcoal, when touched with the platinum loop (Fig. 1, a).

Strain *1074*, when received by us, showed the yeastlike moist appearance (Fig. 1, b) shown by the other strains isolated between 1936 and 1943. According to Berger *et al.* (1) and Berger and Langeron (2), this strain never developed aerial hyphae during the time they observed it, which was over eight years. However,

[1] The author wishes to express his appreciation to Dr. Arturo L. Carrión for his many kindnesses, including the cultures of *T. bergeri* that he sent to him, and to Dr. Carlton Auger of the Institute d'Anatomie Pathologique of the Université Laval of Quebec for the pathological material from the case of Berger and his coworkers.

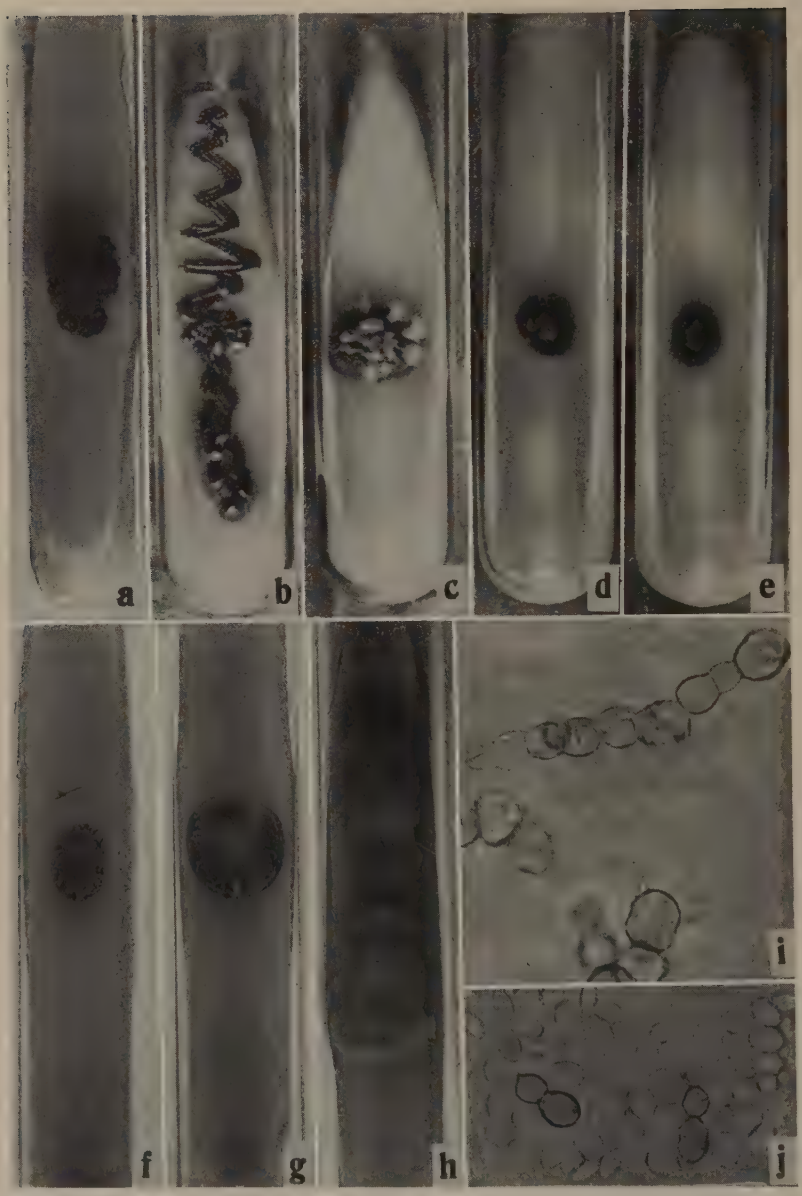

FIG. 1. a, *T. bergeri* No. *1077*. Culture two and a half months old on Sabouraud dextrose agar. b–h, *T. bergeri* No. *1074*. b, c. Cultures five weeks old on Sabouraud maltose agar. d. Culture five weeks old on corn-meal agar, taken from a sector with aerial hyphae. e. Culture five weeks

in one of the first transfers we made, we observed the formation of a sector which had short aerial hyphae. In subsequent transfers to Sabouraud dextrose and maltose agars from the sector with true aerial mycelium, the number of these sectors increased gradually (FIG. 1, c). When we made subcultures from the moist portion of the colony, the same appearance was maintained, while the cultures from the sectors with aerial hyphae became more and more moldy (FIG. 1, f, g, h). The growth in corn-meal agar was always slower than in the aforementioned media and the colonies were almost exclusively made up of submerged pseudomycelium, whether transferred from sectors with aerial hyphae (FIG. 1, d) or from moist and glabrous portions (FIG. 1, e).

The microscopic aspect of strain *1077* is at present the same as described by Berger *et al.* (1) and Berger and Langeron (2), as can be seen in the photomicrograph (FIG. 1, i). With strain *1074* this did not happen. Moist cultures on several media showed almost exclusively yeastlike cells, some with thicker and pigmented walls (FIG. 1, j), and slide cultures with corn-meal agar gave out pseudomycelium with blastospores similar to the ones observed in the genus *Candida* (FIG. 2, a) as described in papers of the authors mentioned above (1, 2).

The cultures with aerial hyphae showed microscopically a true, septate mycelium, from 1 to 3 μ in diameter with septa every 5 to 17 μ (FIG. 2, b). The slide cultures on Sabouraud maltose agar showed the *Pullularia* type of sporulation (FIG. 2, c) in which the conidia grew directly and pleurogenously on the hypha. It was also observed that, on the tips of the hyphae, spores were formed which remained in clusters. In this case there was no differentiation of the conidiophore, because there were no morphological changes with regard to the rest of the hypha (FIG. 2, d, e, g). Single or 2-celled conidiophores were formed perpendicular to or at an angle with the hyphae, usually immediately before a septum

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old on corn-meal agar, taken from a yeastlike culture. f, g, h. Cultures two and a half months old on Sabouraud dextrose agar. i, *T. bergeri* No. *1077*. Culture four months old on Sabouraud dextrose agar; moniliform chains in which some of the cells show budding and others show septa; lactophenol, × 1000. j, *T. bergeri* No. *1074*. Culture four days old on Sabouraud dextrose agar; some cells show thicker and darker walls, lactophenol, × 1000.

(FIG. 2, f, h), which also showed clusters of conidia at their distal end. These conidiophores were cylindrical or bottle-shaped and they, as well as the hyphal ends which bore conidia, sometimes presented a very short extension or neck (FIG. 2, e, h, j) through which a small portion of protoplasm appeared to come out to form a conidium (FIG. 2, h, j). In some cases it was possible to observe a very slight cuplike broadening (FIG. 2, k). In others, the spores remained connected to the hyphae by very thin filaments which looked like cytoplasmic threads (FIG. 2, g).

Clusters of acrogenous conidia were found not only at the tips of cylindrical hyphae and conidiophores as previously described, but also at the end of moniliform chains of cells (FIG. 2, h, i) that were commonly seen in slide cultures and which were formed from yeastlike cells or spores of the inoculum. Also, some of the terminal cells of these chains presented a very short cylindrical neck (FIG. 2, l).

What we have described above clearly shows the evolution of a fungus from the characteristics of the so-called black yeasts to the filamentous stage with true aerial mycelium. This type of transformation has already been observed by several authors, as pointed out by Skinner, Emmons and Tsuchiya (10).

Berger and Langeron, in their paper on *Torula bergeri* (2), divide the so-called black yeasts into two groups: a) those which have a yeastlike stage at first and are later covered with aerial filaments, and b) those which they call true yeasts, without aerial filaments, and that give out a pseudomycelium which penetrates the medium. They place *Torula bergeri* among the latter group. We have already seen that *Torula bergeri* may produce a true aerial mycelium under certain special conditions which we have not as yet determined. Therefore, this separation, which already seemed somewhat artificial, loses its taxonomic value and we are inclined to agree with the opinion of Hansen and Lindner, who consider, according to Skinner *et al.* (10), ". . . that these black yeasts are but yeastlike growth forms of dematiaceous molds of the type of *Cladosporium*. . . ."

As years go by we see how pathogenic species which were originally classified as "black yeasts" are taken out of this group and given a more appropriate place among the filamentous demati-

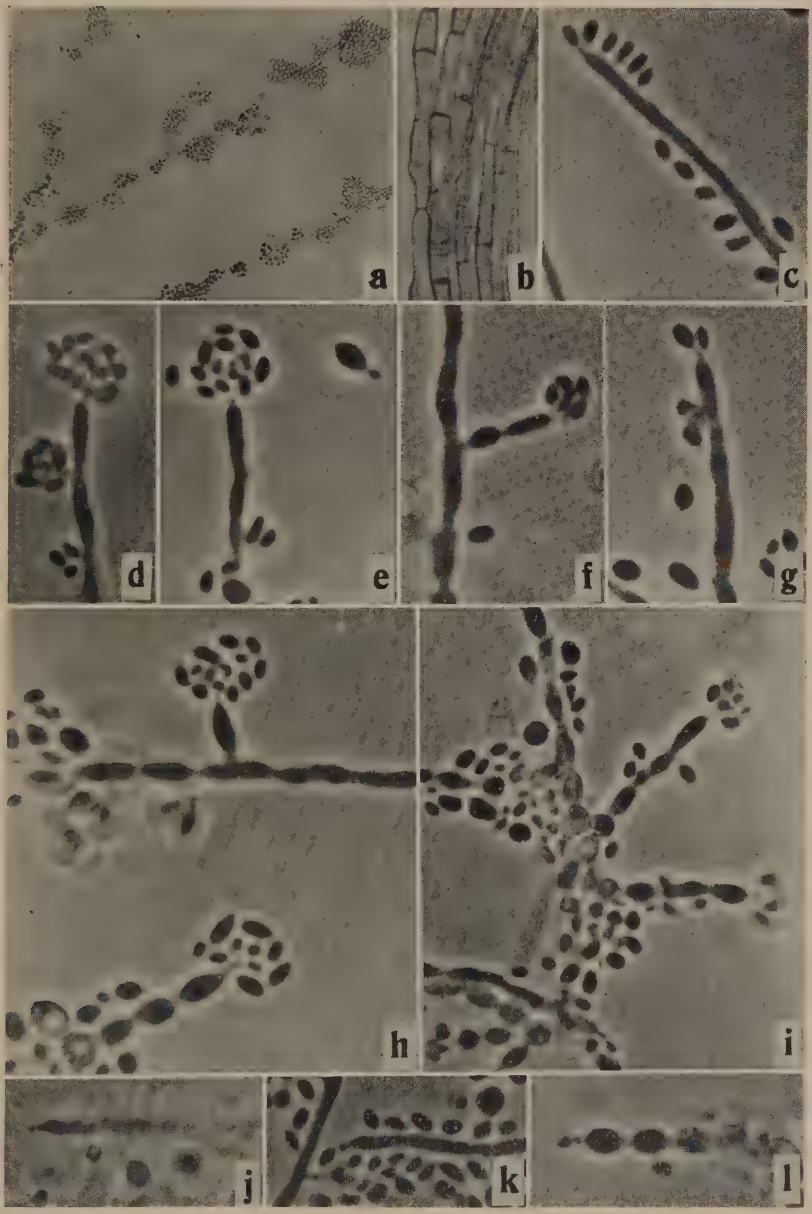

FIG. 2. *T. bergeri* No. *1074*. a. Slide culture 8 days old on corn-meal agar, lactophenol, × 200. b. Culture five months old on Sabouraud glucose agar, lactophenol, × 1000. c–l. Several aspects of slide cultures on Sabouraud maltose agar, 11 days old, lactophenol, phase contrast, × 1200 except i, which is × 1000.

aceous fungi. This has happened with *Torula jeanselmei* Langeron and more recently with *Hormiscium dermatitidis* Kano, which was classified by Carrión (3) as *Fonsecaea dermatitidis* (Kano, 1937) Carrión, 1950. This is now also the case with *Torula bergeri,* to which we have to give a classification more in agreement with the characteristics previously described. Undoubtedly it has a remarkable morphological resemblance with *Phialophora jeanselmei* (Langeron) Emmons, according to the observations made by Emmons (4) and the more recent ones made by Mackinnon *et al.* (7), who have studied in detail the morphology and biology of *P. jeanselmei.* The biological characteristics of *T. bergeri,* as observed by Berger, Beaudry and Gaumond (1), Berger and Langeron (2) and by us, are in agreement with those observed by Emmons (4), Montemayor (8, 9) and Mackinnon *et al.* (7) in *P. jeanselmei.*

We therefore consider *Torula bergeri* synonymous with *Phialophora jeanselmei* (= *Torula jeanselmei* Langeron, 1928 = *Pullularia jeanselmei* (Langeron) Dodge, 1935).

At present four strains of this species are known, the one studied by Langeron in 1928 (6) and the two observed by Emmons (4, 5); all three isolated from mycetomas, and the fourth, called *T. bergeri,* isolated from a case of chromoblastomycosis (1, 2).

We shall publish later on, together with a more detailed comparative study of the strains mentioned above, a discussion of the generic position of this polymorphous and interesting species. We shall also deal with the subject of atypical mycetomas and the atypical chromoblastomycosis of Canada caused by this fungus.

SUMMARY

The author studied *Torula bergeri* isolated by Berger and his coworkers in Canada, and felt that there were no specific differences between this fungus, which produced chromoblastomycosis, and *Phialophora jeanselmei,* one causative agent of mycetomas. Therefore he considers *Torula bergeri* as synonymous with *Phialophora jeanselmei.*

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STUDIES ON SOME SELENOPHOMA SPECIES ON GRAMINEAE [1]

JAI YOUNG PARK [2] AND RODERICK SPRAGUE [3]

(WITH 2 FIGURES)

Selenophoma species are common parasites of the grass family in the temperate and arctic parts of the world. Until recently this group had been little studied and only a few specimens had been collected by mycologists. Sprague and Johnson published information on the morphology of the genus on Gramineae (**8, 9, 10, 11, 12**). Allison confined his detailed investigations to *S. bromigena* (Sacc.) Sprague & A. G. Johnson (**1, 2, 3**). The present paper gives some of the more pertinent data of detailed comparative studies made not only on *S. bromigena* but on *S. donacis* (Pass.) Sprague & A. G. Johnson, *S. everhartii* (Sacc. and Sydow) Sprague & A. G. Johnson and *S. obtusa* Sprague & A. G. Johnson.

MATERIALS AND METHODS

Single-spore pure cultures of *Selenophoma* spp. were obtained from various members of the Gramineae collected in widely scattered parts of the Intermountain West. These cultures were maintained at 20° C. Those actually used in the investigations were limited to one to six isolations obtained from the following collections:

Selenophoma bromigena: on *Bromus inermis* Leyss from Pullman, Washington; *B. ciliatus* L. from Climax, Colorado; *B. carinatus* Hook. & Arn. from Pullman, Washington and Yellowstone Park, Wyoming.

[1] Scientific Paper No. 1071, Washington Agricultural Experiment Stations, Pullman. Project No. 449.

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S. donacis: on *Elymus glaucus* Buckl., Pullman, Washington; *Poa ampla* Merr., Pullman, Washington; *Triticum aestivum* L., Pullman, Rosalia and Colfax, Washington and Moscow, Idaho.

S. everhartii: on *Calamagrostis rubescens* Buckl., Angle Peak, Washington; *Festuca myuros* L., Skyline Drive, Idaho.

S. obtusa: on *Agropyron spicatum* (Pursh) Scribn. & Sm., Angle Peak, Washington; *Elymus glaucus* Buckl., Cedar Pass, California.

Spore germination studies were made of all of these isolates, using 100 spores from each collection. Isolation onto potato-dextrose agar was done by means of a dry glass micropipette. Studies were conducted at temperatures of 1, 6, 10, 15, 20, 25 and 30° C. Spore germination and mycelium growth rate were compared at several different pH levels, ranging from 3.0 to 8.0. The pH of the media was adjusted with HCl and NaOH, using a Beckman Electric pH meter for recording.

Nuclear division in the germinating spores of *S. donacis* from wheat collected at Pullman, Washington, was studied at 20° C. Transfer was made in the same way as in the spore germination studies. The spores that were examined were stained with Heidenhain's iron haematoxylin from 5 to 25 hours after isolation.

Relative humidity studies were made by growing the 100 samples of dry spores on a glass slide in air bubbled through various solutions to maintain the following approximate percentages of relative humidity: 100 % (water only), 95% (sodium sulfite), 90% (zinc sulfate), 81% cent (ammonium sulfate) and 76% (sodium chlorate) relative humidity (**5**, **6**, **7**).

Cross inoculation studies were made in the greenhouse and out-of-doors by spraying seedlings, which were 20 to 30 days old, with pycnospore suspensions obtained from the various pure cultures listed above. The most satisfactory spray chamber was a wooden box with dimensions of $3 \times 2 \times 2.5$ feet, embedded in the ground and covered with a muslin cloth. Humidity was maintained by keeping a six-inch floor layer of peat moss dampened with water. At a favorable temperature of 65–70° F. the incubation period was about 72 hours. No artificial light was used. Allison (**3**), among others, found that spores of *S. bromigena* germinated readily in the dark.

Forty-three species of Gramineae in fifteen genera were used in cross-inoculation trials. These are shown in Tables II and III.

The data obtained in these studies are given in detail in an unpublished manuscript by the senior writer [4] filed at Washington State College. The present paper briefly summarizes most of these data plus some additional information.

EFFECT OF TEMPERATURE

Spores of most of the species germinated in the temperature range of 15–25° C. within 20 to 30 hours after isolation. Within 40 hours from 60 to 100 per cent of the spores had germinated. At 30° C. all of the fungi were inhibited, only 12 to 20 per cent of the spores germinating after 40 hours. Most of the fungi had a minimum temperature for spore germination of 5–6° C. However, some spores of *S. bromigena* from *Bromus inermis* and *S. donacis* from *Poa ampla* did not germinate at temperatures below 10° C. after 40 hours incubation.

The largest number of spores of *S. everhartii* from *Calamagrostis rubescens* and *Festuca myuros* germinated at 25° C. while the isolates of *S. donacis, S. bromigena* and *S. obtusa* tended to have their maximum germination at 18–22° C. Allison (3) reported that *S. bromigena* had an optimum temperature of 19–22° C. for spore germination with the minimum for spore germination between 3° C. and 5° C.

The relationship between hyphal production and secondary conidial production was compared at temperatures of 1, 6, 10, 15, 20, 25 and 30° C. (Table I). In general, the production of secondary conidia increases with an increase in temperature. At 20° C. or higher *S. donacis* from wheat and *Poa ampla* and *S. bromigena* from *Bromus carinatus* produced masses of secondary conidia (pseudopionnotes) from germ tubes without production of hyphae. The mass of conidia were formed at the septate portion of the germ tube (FIG. 1, D).

[4] Jai Young Park. Comparative Studies of Selenophoma Leaf Spot Diseases on Certain Grasses. Thesis submitted to Washington State College as partial fulfillment of the requirements for the degree of Doctor of Philosophy. The author expresses his deep appreciation of the aid of George W. Fischer, Geneva Fischer, and C. Gardner Shaw in the preparation of this thesis.

TABLE I

THE RELATIVE AMOUNT OF HYPHAE (−) AND CONIDIA (+) PRODUCED UNDER DIFFERENTIAL TEMPERATURE RANGE ON POTATO DEXTROSE AGAR AT 20° C. AFTER 70 HOURS' INCUBATION

Isolate/Host	Temperature range (°C.)						
	1	6	10	15	20	25	30
Selenophoma bromigena[5] *Bromus inermis*		−−+	−−++	−−++	−+++	−−−−	−−−−
Selenophoma bromigena *Bromus carinatus*	−	−−	−−++	−+++	++++	++++	++++
Selenophoma donacis *Triticum aestivum*		−−	++	+++	+++	++++	++++
Selenophoma donacis *Poa ampla*	−	−−	−++	−+++	++++	++++	++++
Selenophoma everhartii *Festuca myuros*		−−	−−−+	−−−+	++−−	−−−−	−−−−
Selenophoma everhartii *Calamagrostis rubescens*		−−	−−+	−−−+	++−−	−−−−	−−−−
Selenophoma obtusa *Agropyron spicatum*		−−	−−−+	−−−+	++−−	−−++	−−++

[5] Relative amount of hyphae (−) or conidia (+) shown by number of dashes (−) or plus marks (+)

In general, relative development of hyphal production increased at lower temperatures as, inversely, conidial production fell off. Some cultures of *S. bromigena* from *Bromus inermis* from Pullman produced somewhat aberrant conidia at temperatures as low as 6° C.

COMPARATIVE MORPHOLOGY OF THE GERMINATING SPORE

There was considerable variation in the process of spore germination in the several isolations. Similar results are reported in the literature. Darley (**4**) reported that septation occurs in the pycnospore before germination on nutrient media but not in distilled water. In our trials pycnospores of *S. bromigena* from *Bromus inermis* on potato-dextrose agar produced germ tubes sometimes after one septum was formed in the spore, or in some cases before any septation had formed. These germ tubes developed from any portion of the pycnospore about 10 hours after incubation at 20° C. Allison (**1**) reported that germ tubes formed on either end of the spores after 12 hours.

Sometimes in *S. bromigena* from *B. inermis* one or two oval nodes are formed at the terminal portion of the germ tube after 20

hours (FIG. 1, A). Sometimes digitately branched hyphae occur after 25 hours (FIG. 1, B). These hyphae increase in number, forming a cluster which is a pycnidial initial.

There were some differences noted among some of the isolates. For instance, *S. bromigena* from *Bromus carinatus* developed three or four septa in the spore at the start of germination (FIG. 1, C). The hyphae from these germinating spores formed numerous cross walls. In the case of *S. everhartii* from both *Calamagrostis* and *Festuca* the spores germinated without previous septation.

In *S. obtusa* from *Agropyron spicatum* the spores became conspicuously swollen within an hour. Germ tubes were formed from the middle portion of the spores or from both ends after about 12 hours incubation at 20° C.

EFFECT OF ATMOSPHERIC HUMIDITY ON SPORE GERMINATION

The spores of all isolations grew well at 100% relative humidity and appreciably less at lower humidities. At 81% humidity the growth was very slow. Allison (3) suggested that a film of water on the leaves at the time of infection and continued high humidity was necessary for rapid development of *S. bromigena* on *Bromus inermis*. This is indicated by our results.

EFFECT OF pH ON SPORE GERMINATION AND COLONY GROWTH

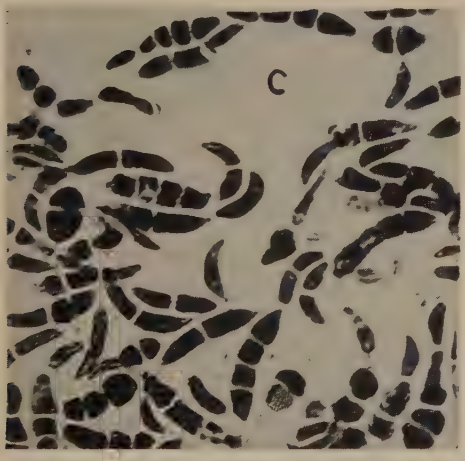
With few exceptions, spores of all species of *Selenophoma* germinated well at p ranges of 4.5–8.0, with the optimum at 5.0–6.0. At pH 3.0 spore germination decreased sharply.

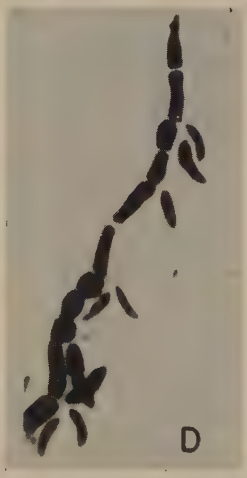
Colonies of all species grew well at pH 4.0–8.0 with the optimum for all except *S. bromigena* at pH 5.5. *S. bromigena* from all hosts had an optimum range of pH 4.5–5.0.

FIG. 1. Germinating spores and pure cultures of *Selenophoma*. A. Spore germination of *S. bromigena* from *Bromus inermis*, 12 hours after isolation, ×700. B. *S. bromigena* from *B. inermis* showing digitate branching of hyphae, 25 hours after isolation, ×750. C. Germinating spores of *S. bromigena* from *Bromus carinatus* showing multiple septation 15 hours after isolation, ×750. D. Showing secondary conidial formation in *S. donacis* from *Agropyron cristatum*, 24 hours after isolation, ×750. E. Pure culture of *S. bromigena* on potato-dextrose agar, Race 1. F. Pure culture of *S. bromigena*, Race 2.

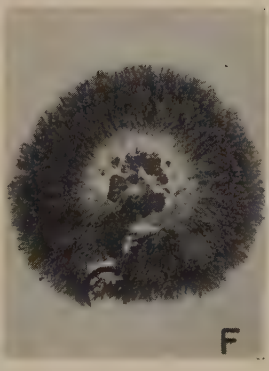
A

B


C


D

E


F

ADDITIONAL INFORMATION ON MACROSCOPIC APPEARANCE OF THE COLONIES

Sprague and Johnson (12) and Allison (3) have detailed information on the general appearance of isolations of most of the fungi handled in the current paper. Such data will not be repeated here.

It was found that a relatively high humidity could be maintained by sealing the petri dish cultures with rubber band seals. All of the fungi studied were slow growing, requiring two months to reach the margin of a 100 mm. petri dish at 20° C.

S. bromigena isolated from *Bromus carinatus* had a somewhat more carbonaceous appearance than those from *B. inermis* (compare FIGS. 1, E and 1, F). Isolates of *S. bromigena* from *B. ciliatus* from Climax, Colorado resembled those from *B. inermis* from the Palouse region and from Minnesota (3).

Isolates of *S. everhartii* from *Festuca myuros* produced flat, flesh-tinted, subcottony colonies with several rings of purple-brown to creosote-brown color at the margins and with strongly tinted vinaceous substrata. The small, black spore masses formed in the central area of the colony. Some colonies isolated from the same material had a flat, smooth surface of burnt-orange with no subcottony overgrowth. The first-mentioned type of colony resembles isolates of *S. everhartii* obtained from *Trisetum spicatum* (L.) Richt. by Sprague and Johnson (12) while the burnt-orange kind is similar to colonies of *S. everhartii* obtained from *Deschampsia danthonioides* (Trin.) Munro (12). The isolates obtained from *Calamagrostis rubescens* resembled these also except that the burnt-orange colonies from *Calamagrostis* developed a thin overgrowth of white mycelium.

GROWTH RATES OF THE SELENOPHOMA CULTURES ON POTATO-DEXTROSE AGAR

Experience with this group has shown that unless all isolates have been subjected to the same period of time in culture since their original wild state the comparative rate of growth of colonies of the same species may vary appreciably. While the studies herein briefly reported have avoided this to a considerable extent, we are still unsatisfied that all the differences noted are significant. However, in

general, *S. obtusa* grew almost twice as fast as isolates of *S. everhartii*. In a period of seven weeks the isolates of *S. obtusa* from *Agropyron spicatum* averaged 75 mm. in diameter while those of *S. everhartii* from *Festuca* and *Calamagrostis* averaged 33–35 mm. Cultures of *S. bromigena* and *S. donacis* grew at an intermediate rate of 47–56 mm. diameter in the same period of time.

PATHOLOGICAL HISTOLOGY

This work was limited to *S. bromigena* invading inoculated leaves of *Bromus inermis* and *B. carinatus*. The results confirmed, in the main, the detailed study by Allison (3). In the present studies the host penetration was through the stomatal openings. Sometimes masses of hyphae accumulate on the surface of the closed stomatal opening, invading the host after the stoma opened.

NUCLEAR BEHAVIOR DURING SPORE GERMINATION

The spores of *Selenophoma donacis* from *Triticum aestivum* were isolated on potato-dextrose agar at 20° C. and the nuclear division in the germinating spore was observed during the period of germination.

The spores were removed and stained with Heidenhain's haematoxylin, at 5–10, 10–15, 15–20 and 20–25 hours after they had been isolated on potato-dextrose agar. Nuclear division took place 5–10 hours after isolation (FIG. 2, A, B) and a septum developed between the two nuclei in the spore. One of these nuclei re-divided and at the same time a germ tube formed from the terminal portion of the spore 10–15 hours after isolation. One of the newly divided nuclei moved into the germ tube but the other nucleus remained in the spore body (FIG. 2, C). The nucleus in each half of the spore divided at the same time or about 15–20 hours after isolation (FIG. 2, D), and a germ tube resulted from each end of the spore. Further development of nuclei is shown in FIG. 2, E.

RESULTS OF INOCULATIONS

The results of cross-inoculation trials are partly shown in TABLES II and III. In addition to the inoculation results given in TABLES

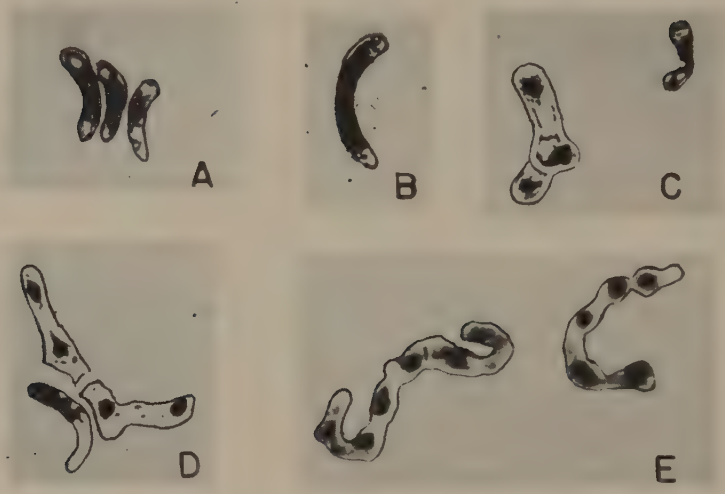


FIG. 2. The nuclear behavior during spore germination of *Selenophoma donacis* from *Triticum aestivum*. A. About 5–10 hours after isolation. B. Ten hours after isolation. C. 10–15 hours after isolation. D. 15–20 hours after isolation. E. 20–25 hours after isolation.

II and III, the following species were all inoculated with spore suspensions of the fungi listed in TABLES II and III and with entirely negative results: *Agropyron inerme* (623 leaves), *A. repens* (697 leaves), *A. sibiricum* no. *27* (1006 leaves), *Arrhenatherum elatius* no. *492* (804 leaves), *Bromus vulgaris* no. *2640* (966 leaves), *Festuca pacifica* (941 leaves), *Phalaris arundinacea* (688 leaves), *Phleum pratense* (776 leaves), *Poa compressa* (977 leaves), *Sitanion hystrix* no. *5505* (577 leaves), *Stipa columbiana* no. *1703* (761 leaves), *Stipa comata* no. *4903* (662 leaves) and *Stipa viridula* no. *3085* (518 leaves).

Most of the results which are listed above and in TABLES II and III are self-evident. The great majority of the isolates were restricted to their original hosts or to closely related ones. There were a few exceptions. *S. donacis* from *Triticum aestivum* attacked *Poa pratensis* but *S. donacis* from *Poa ampla* did not attack *Triticum*. Sprague (8) previously reported flecking on *Poa pratensis* from *S. donacis* from wheat from the Palouse region. This suggests one possible source of the occasional outbreak of *Selenophoma* on wheat in the Palouse region of Washington and Idaho. The iso-

late of *S. donacis* from *Elymus* is apparently a distinct race from that on wheat and *Poa* because it was confined to *Elymus* with only flecking on wheat and it was unable to attack *Poa* spp. The race on wheat in the Palouse region is called Race 1, that on *Poa ampla* at Pullman Race 2, and that on *Elymus* at Pullman is Race 3. The fact that Race 1 is able to attack *Poa pratensis* indicates affinity to Race 2, but Race 2 did not attack *Poa pratensis* in the current trials. All of these races appear at least as clear-cut as the considerably more minutely segregated ones of stem rust (*Puccinia graminis* Pers.).

Isolates of *S. bromigena* from *B. inermis, B. carinatus* and *B. ciliatus* were confined to brome grasses in our studies. The isolates from *Bromus carinatus* did not parasitize most collections of *B. inermis* except nos. *5792* and *8024* which were mildly attacked. The "carinatus" strain appears to be a relatively weak race of *S. bromigena*. It attacked *B. carinatus* in only 26 out of 148 leaves. The race on *B. inermis* would not attack true *B. carinatus* but it did attack one strain each of *B. marginatus* and *B. polyanthus* which are almost identical with *B. carinatus*. Strangely enough, the weak race on *B. carinatus* caused injury on *B. brizaeformis* and on *B. tectorum* while the race from *B. inermis* did not attack it. The common race in the Palouse on *B. inermis* is called Race 1 of our series and the uncommon race on *B. carinatus* is Race 2. Perhaps it only deserves a subclass, such as Race 1-B; but on the strength of our present available data, it seems simpler to classify it as a distinct race. *Selenophoma* on *B. carinatus* is uncommon in the West but seems to be increasing slowly. Allison (3) did not obtain infection on this host with material from *B. inermis,* indicating that the material of his more parasitic strain was comparable to our Race 1. The isolate from Colorado material of *B. ciliatus* possibly should be left unclassified because no available seed of *B. ciliatus* would germinate. On the other hosts it only weakly attacked the *5792* strain of *B. inermis*. Since it did not attack *B. marginatus* no. *10,708* as did Race 2, it would appear that it was another weak Race and can be called Race 3.

Selenophoma obtusa isolated from representatives of the two closely related genera *Agropyron* and *Elymus* were confined to

TABLE II

RESULTS OF CROSS-INOCULATION EXPERIMENTS WITH SELENOPHOMA SPP. ON VARIOUS GRASSES

Plant inoculated	Number of leaves infected (+) or not infected(−) by:											
	Selenophoma bromigena						*Selenophoma donacis*					
	Isolated from *Bromus inermis* (7)[2]		Isolated from *Bromus carinatus* (2)		Isolated from *Bromus ciliatus* (1)		Isolated from *Poa ampla* (3)		Isolated from *Elymus glaucus* (4)		Isolated from *Triticum aestivum* (5)	
	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)
Agropyron cristatum	0	54	0	38	0	32	—	—	—	—	—	—
Agropyron spicatum 2719, 737, 6409[1]	0	141	0	141	0	104	0	154	0	123	0	88
Agropyron trachycaulum	0	79	0	56	0	77	0	56	0	34	0	65
Bromus anomalus 8027	0	136	0	181	0	76	0	34	—	—	—	—
Bromus brizaeformis	0	256	34	20	0	67	0	86	0	25	0	38
Bromus carinatus	0	232	26	122	0	89	0	35	0	47	0	49
Bromus erectus 4684	0	207	18	154	0	38	—	—	—	—	—	—
Bromus erectus 2336	19	169	0	193	0	32	—	—	—	—	—	—
Bromus inermis 3053	0	321	0	213	0	82	0	36	0	42	0	25
Bromus inermis 3197	74	169	0	101	0	65	0	42	0	36	0	45
Bromus inermis 5792	104	74	20	116	8	98	0	24	0	37	0	49
Bromus japonicus 1733	0	187	0	131	0	131	0	89	0	34	0	43
Bromus marginatus 8040	27	141	0	243	0	74	0	38	0	64	0	49
Bromus marginatus 5355	6	178	0	121	0	89	0	21	0	87	0	96
Bromus marginatus 2133	0	210	0	262	0	66	0	32	0	67	0	81
Bromus marginatus 3972	44	274	0	281	0	59	0	65	0	87	0	36
Bromus marginatus 7253	26	58	0	134	0	67	0	37	0	98	0	62
Bromus marginatus 10,708	0	282	28	144	0	97	0	34	0	88	0	48
Bromus marginatus 11,206	0	198	0	201	0	145	0	29	0	65	0	77
Bromus marginatus 8024	30	123	22	74	0	83	0	63	0	55	0	98
Bromus mollis 1728	0	227	0	242	0	87	—	—	—	—	—	—

TABLE II—*Continued*

Plant inoculated	Number of leaves infected (+) or not infected (−) by:											
	Selenophoma bromigena						*Selenophoma donacis*					
	Isolated from *Bromus inermis* (7)[2]		Isolated from *Bromus carinatus* (2)		Isolated from *Bromus ciliatus* (1)		Isolated from *Poa ampla* (3)		Isolated from *Elymus glaucus* (4)		Isolated from *Triticum aestivum* (5)	
	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)
Bromus polyanthus 7477	17	93	0	221	0	98	0	36	0	67	0	34
Bromus tectorum	0	287	8	14	0	45	0	21	0	35	—	—
Bromus tomentellus 2447	0	285	0	191	0	52	0	21	0	21	0	31
Calamagrostis epigeios (L.) Roth.	0	22	0	47	0	43	0	32	0	39	0	54
Dactylis glomerata 8775	0	213	0	245	0	88	0	59	0	121	0	110
Elymus canadensis 3889	0	81	0	89	0	39	0	121	6	50	0	89
Elymus glaucus 2662	0	92	0	49	0	19	0	187	28	12	0	112
Festuca elatior 3161	0	123	0	201	0	36	0	43	0	98	0	38
Festuca ovina	0	98	0	56	0	39	0	59	0	49	0	56
Festuca pacifica	0	145	0	167	0	22	0	151	0	65	0	33
Hordeum vulgare	0	111	0	121	0	39	—	—	—	—	—	—
Hordeum vulgare	0	39	0	36	0	11	0	39	0	39	0	42
Hordeum vulgare	0	221	0	131	0	49	—	—	—	—	—	—
Hordeum vulgare	0	225	0	312	0	39	0	41	0	47	0	33
Poa ampla	0	138	0	203	0	34	21	40	0	87	0	73
Poa pratensis 3128	0	65	0	34	0	32	0	136	0	81	40	64
Secale cereale	0	113	0	289	0	89	0	76	0	58	0	39
Secale cereale	0	210	0	149	0	33	—	—	—	—	—	—
Stipa thurberiana 3710	0	18	0	56	0	14	0	36	0	34	0	59
Triticum aestivum	0	213	0	311	0	49	0	55	0[3]	69	44	40

[1] These numbers are the accession numbers of the Pullman (Wash.) Nursery Unit of the Soil Conservation Service, United States Department of Agriculture, whose aid in furnishing seed for these trials was invaluable.

[2] The figures in parentheses refer to number of pure culture isolations that were used in the trials.

[3] Flecking only.

TABLE III

RESULTS OF CROSS-INOCULATION EXPERIMENTS WITH SELENOPHOMA SPP. ON VARIOUS GRASSES

Plant inoculated	Number of leaves infected (+) or not infected (−) by:							
	Selenophoma obtusa				*Selenophoma everhartii*			
	Isolated from *Agropyron spicatum* (1)		Isolated from *Elymus glaucus* (1)		Isolated from *Festuca myuros* (1)		Isolated from *Calamagrostis rubescens* (1)	
	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)
Agropyron cristatum	0	115	0	87	0	56	0	76
Agropyron spicatum 2719	36	35	0	124	0	89	0	45
Agropyron spicatum 737	0	145	0	113	0	53	0	79
Agropyron spicatum 6409	0	89	0	48	0	49	0	39
Agropyron trachycaulum	0	115	54	112	0	66	—	—
Bromus anomalus 8027	—	—	—	—	—	—	—	—
Bromus brizaeformis	0	26	0	47	0	78	—	—
Bromus carinatus	0	87	0	38	0	37	0	34
Bromus erectus 4684	0	34	0	81	0	59	0	46
Bromus erectus 2336	—	—	—	—	—	—	0	34
Bromus inermis 3053	0	19	—	—	0	26	—	—
Bromus inermis 3197	0	56	—	—	0	45	—	—
Bromus inermis	0	118	0	201	0	46	—	—
Bromus japonicus 1733	—	—	—	—	—	—	0	56
Bromus marginatus 8040	0	47	—	—	0	78	—	—
Bromus marginatus 2133	—	—	—	—	0	45	0	89
Bromus marginatus 3972	0	49	—	—	0	86	—	—
Bromus marginatus 7253	0	87	0	69	0	82	0	69
Bromus marginatus 10,708	0	32	0	45	0	96	0	56
Bromus marginatus 11,206	0	53	0	64	0	62	0	63
Bromus marginatus 8024	0	53	0	59	—	—	—	—
Bromus mollis 728	0	24	0	39	—	—	—	—
Bromus polyanthus 7477	0	39	0	89	0	47	0	88
Bromus tectorum	0	56	0	26	—	—	—	—
Bromus tomentellus 2447	0	29	—	—	—	—	—	—
Calamagrostis epigeios	0	55	0	23	0	118	8	28
Dactylis glomerata	0	92	0	54	0	79	0	24
Elymus canadensis	10	38	0	54	0	54	0	82
Elymus glaucus	0	43	45	236	0	27	0	42
Festuca elatior	0	34	0	37	0	42	0	90
Festuca ovina	0	44	0	41	10	42	0	69
Hordeum vulgare	0	65	0	67	—	—	—	—
Hordeum vulgare	0	45	0	39	—	—	—	—
Hordeum vulgare	0	23	0	43	—	—	—	—
Hordeum vulgare	—	—	0	13	—	—	—	—
Poa ampla	0	123	0	76	0	88	0	42
Poa pratensis	0	97	0	92	0	18	0	87
Secale cereale	0	67	0	54	—	—	—	—
Secale cereale	0	78	0	34	—	—	—	—
Stipa thurberiana	0	23	—	—	0	43	0	23
Triticum aestivum	0	65	0	98	—	—	—	—

these genera in their host range (TABLE III). The isolate from *Agropyron* could attack its original host and also *E. canadensis*. The isolate from *Elymus* could attack *A. trachycaulum* and its original host. The results do not indicate any racial difference in these two isolations; they may be considered as one strain.

The somewhat scanty trials with *S. everhartii* indicated that the isolate from *Festuca myuros* was confined to fescue and the isolate from *Calamagrostis* to that genus.

SUMMARY

The spores of most species of *Selenophoma* have an optimum temperature range for germination of 15–25° C. and a minimum of 6° C. except *Selenophoma bromigena* on *Bromus inermis* which germinated appreciably at 1° C. *Selenophoma everhartii* is the species best adapted to high temperatures of any of the graminicolous *Selenophoma* that were studied. Its optimum temperature for spore germination was 25° C. Its spores germinated slowly, requiring 40 hours' incubation to reach maximum spore germination.

Most species and varieties of *Selenophoma* produced some secondary conidia as well as hyphae at temperatures of 15–20° C. However, the following isolates characteristically produced only hyphae, without conidia, at 25° C. or higher temperatures: *Selenophoma bromigena* from *Bromus carinatus* and *S. everhartii* from *Festuca myuros,* and *Calamagrostis rubescens.*

In different stages of development starting with pycnospore germination, *Selenophoma bromigena* from *Bromus inermis* produced one or two oval nodes at the terminal portion of the germ tube (20 hours after isolation), the characteristic palm-shaped branching hyphae (25 hours later), and then hyphal cluster formation. This species also produced the most abundant hyphae of any of the species or varieties of *Selenophoma. S. bromigena* from *B. carinatus* was distinguished by the formation of several septations in the spore before germination.

In general, all species and varieties of *Selenophoma* germinated most rapidly at 100% relative humidity, but slower germination occurred even down to 81% humidity.

Most of the species and varieties of *Selenophoma* had an optimum pH of 5.0–6.0. However, *Selenophoma bromigena* had optimum ph ranges of 4.5–5.0.

S. obtusa on *Agropyron spicatum* grew more rapidly and vigorously than any of the isolates, growing approximately two times faster in diameter of the colony than *S. everhartii* which is the slowest growing fungus in the genus *Selenophoma* on Gramineae.

Selenophoma bromigena from *Bromus inermis* and *B. carinatus* penetrated through the stomatal chamber in all cases by forming appressoria. The mycelium grew intercellularly. The hyphae in the subcuticular region formed secondary pycnidia. Pycnidia appeared two weeks to 20 days after inoculation.

In *Selenophoma donacis* on *Triticum aestivum* nuclear division took place 5 to 10 hours after isolation on potato-dextrose agar, and a septum developed between the two nuclei of the spore. One of these nuclei redivided and at the same time a germ tube formed. The germ tube developed from the terminal portion of the spore, 10 to 15 hours after isolation.

The optimum temperature range for the infection of hosts by spore suspensions was 65–70° F., with an incubation period of 72 hours. The host plant was most susceptible when the 20–30-day-old plant was 3–4 inches tall. A subterranean inoculation chamber was the most satisfactory equipment used in cross inoculation studies.

Selenophoma bromigena did not attack any hosts other than members of the genus *Bromus*. It had three races, number one on *Bromus inermis*, a weakly pathogenic Race 2 on *Bromus carinatus* and another weak one, Race 3, on *B. ciliatus*.

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MICROMYCES AND SYNCHYTRIUM

John S. Karling

The genus *Micromyces* was established by Dangeard in 1888 for a minute chytrid parasite which he found in the alga *Zygogonium* and named *M. zygogonii*. This and other similar parasites of the Conjugatae were observed earlier by Thwaites (**1846**), Shadbolt (**1852**), Smith (**1853**), Pringsheim (**1857**), deBary (**1858**) and Reinsch (**1875, 1879**), but these workers were not certain of the parasitic nature of these organisms and described the spiny prosori and resting spores as asteridia and astrospheres. Dangeard recognized the close similarity of *Micromyces* to the older and better known genus *Synchytrium* (deBary and Woronin, **1863**), but because of the former's minute size, aquatic habitat and its formation of a sporangial sorus outside of the initial cell he regarded it as a distinct and valid genus. *Micromyces zygogonii* was subsequently reported by Dangeard (**1891**), de Wildeman (**1891**), Petersen (**1909, 1910**), Minden (**1911**), Denis (**1927**), Huber-Pestalozzi (**1931**), Heidt (**1937**), Couch (**1931, 1937**), Sparrow (**1943**), Canter (**1949**) and Rieth (**1950A**), and five new species and one variety were added to the genus by Scherffel (**1904**), Skvortzow (**1925**), Couch (**1937**), Sparrow (**1943**), Canter (**1949**), and Rieth (**1950B**).

Most of these workers recognized the fundamental similarity of *Micromyces* to *Synchytrium*, and as early as 1892 Fischer noted that *Micromyces* should be placed in his new family Merolpidiaceae (Synchytriaceae) if Dangeard's (**1888**) observations were confirmed. He further stated (p. 72): "Er wurde der Sectio *Pycnochytrium* der Gattung *Synchytrium* entsprechend, wo ebenfals die allein vorhanden Dauersporen bei der Keimung einen Sporangiensorus liefern." Schroeter (**1897**) failed to mention *Micromyces* in his monograph on the Chytridineae, but de Wildeman (**1900**) expressed the same viewpoint as Fischer regarding the taxonomy of the genus. Its fundamental similarity to *Synchytrium* was again

emphasized by Scherffel (1926) who stated, "Die Übereinstimmung zwischen einem *Pyknochytrium* und *Micromyces* ist überhaupt eine derart vollkommene, dass es sich fragt, ob die weitere Aufrechterhaltung der Gattung *Micromyces* zulassig ist." Couch (1931) likewise pointed out that the life cycle, development and cytology of *Micromyces* species are strikingly like those of *Synchytrium* species whose sporangial sori develop as an extrusion or outgrowth of the mature thallus or initial cell (*Mesochytrium*). To quote Couch (1931) who gave an adequate comparison of the two genera as they were known at that time:

"In comparison with *Micromyces* the prosori and resting bodies of *Synchytrium* are much larger, reaching a maximum size in the former genus of 33.6 μ and in the latter of 180 μ or more. In *Synchytrium* the prosori and resting spores are seldom if ever spiny. The resting spores, however, are often covered with a thick warted membrane, or the membrane may be coarsely reticulated as in *S. endobioticum* or *S. saxifragae*. The prosorus contains a single large nucleus which in *S. endobioticum* attains the enormous diameter of 25 μ. The nucleus in the prosorus of the present species of *Micromyces* is 8 μ thick, a comparatively large nucleus for such a small thallus. In the subgenus *Mesochytrium* a thin-walled vesicle is extruded from the prosorus and the protoplasm flows into this and is then delimited into sporangia. In *Micromyces zygogonii* the sorus is formed in precisely the same way. The number of sporangia contained in one sorus in *Synchytrium* is often up to 100 or even more, rarely 5–10 (*S. endobioticum*). In *Micromyces* the number varies from 3–24. The swarmspores are uniciliate and vary from 3–6 μ thick; in *Micromyces* they are about 1 μ thick. Curtis (1921) has shown that in addition to swarmspores gametes also are formed which fuse in pairs to form a biciliate zygote and from this zygote the resting spore is formed. The gametes except for their behavior are indistinguishable from the swarmspores. In the species of *Micromyces* here described I have found an apparent fusion of gametes similar to that described by Curtis. In short the cytology and development of the present species of *Micromyces* and *Synchytrium* as illustrated by *S. endobioticum* are strikingly similar. But in view of the slight morphological differences between the two genera and the great difference in size correlated with a striking difference in habitat it seems best to retain the genus *Micromyces* rather than merge it with the sub-genus *Mesochytrium*."

The species referred to above as *M. zygogonii* was later found to be a different species which Couch (**1937**) named *M. longispinosus.*

Another difference, which was not emphasized by Couch, is that in *Micromyces* the young newly-entered parasite may be amoeboid in movement and develop pseudopodia which are frequently retracted and formed anew (Canter, **1949**). Such changes in shape and motion have not been reported for *Synchytrium*, although young thalli apparently move or are moved to the base of the host cell as in *S. endobioticum*. Also, in fixed and stained preparations they may appear somewhat irregular in outline and lie close to the host nucleus as in species of *Micromyces*. *Micromyces* apparently does not form a common and distinct sorus membrane which independently envelops all of the sporangia as in *Synchytrium*. The descriptions of most students of *Micromyces* are not certain on this point, and their drawings do not indicate that the sporangia are liberated by the rupture of a separate sorus wall. However, in *M. ovalis* Rieth (**1950B**) has figured and described the sorus membrane as rupturing or breaking only at the points of contact of the four cleavage segments or sporangia. The four segments of the sorus membrane become, thus, the outer wall of the sporangia.

However, this difference, together with the amoeboid movement of the thallus in *Micromyces*, differences in size, spinyness of the resting spores, habitat and hosts are not generically significant in the author's opinion. They are outweighed by the fundamental structural and developmental similarities of the two genera. There are no valid reasons, so far as our knowledge goes at present, for retaining *Micromyces* as a separate genus, and it is herewith merged with *Synchytrium*. Its species are accordingly renamed **S. zygogonii** (Dang.) comb. nov., **S. petersenii** (Scherff.) comb. nov., **S. spirogyrae** (Skvort.) comb. nov., **S. longispinosus** (Couch) comb. nov., **S. laevis** (Canter) comb. nov., and **S. ovalis** (Rieth) comb. nov. De Wildeman's (**1900**) *Micromyces mesocarpi* is obviously a species of *Micromycopsis* and need not be considered here.

Couch emphasized the close resemblance of *Micromyces* to the subgenus *Mesochytrium* of which *S. endobioticum* is regarded as a typical member. However, in this species the resting spore func-

tions as a sporangium in germination and gives rise directly to zoospores. In the former *Micromyces* species (*S. zygogonii, S. longispinosus* and *S. laevis*) the resting spore functions as a prosorus in germination by extruding a thin-walled vesicle into which the protoplasm flows and later segments into sporangia. Therefore, on this basis they do not belong in *Mesochytrium* or any of the other known subgenera. They have the same life cycle and sequence of development as *S. fulgens,* with the exception that in the latter species the mature thallus functions as a sorus. Fusion of motile isogametes to form a zygote has been reported in both species, although the present-day evidence of sexuality in *S. longispinosus* is not as convincing as for *S. fulgens.*

In the latter species sexual reproduction occurs so generally that Kusano considered it to be typical and asexual reproduction as accessory. In *S. longispinosus,* on the other hand, only fusing gametes and biflagellate zygotes, which later penetrated the host cell, have been observed. Couch (**1931**) was unable to follow the development of the zygotes into mature resting spores.

In view of the fact that former *Micromyces* species do not fit into any of the known subgenera a new one, *Microsynchytrium,* is proposed to include them. This name was chosen because the species included in the subgenus at present are very minute in comparison with most members of the other subgenera. Germination of the resting spores in *S. spirogyrae, S. petersenii* and *S. ovalis* has not been observed, and their inclusion in *Microsynchytrium* at present is open to question.

Furthermore, in light of Kusano's (**1930**) intensive study of *S. fulgens* and discovery that the resting spores function as prosori in germination it is obvious that this species also does not belong to any of the known subgenera. Accordingly, another subgenus, *Exosynchytrium,* is proposed for it and other species which may be found in the future to have the same life cycle and type of development. This subgenus is placed near *Eusynchytrium* in the same sense that *Microsynchytrium* is close to *Mesochytrium.* It is quite probable that other known species of *Synchytrium* will fit into either of these subgenera when their full life cycles become known.

Two other genera of aquatic algal parasites, *Micromycopsis* and *Endodesmidium*, are included in the Synchytriaceae and appear to be closely related to *Synchytrium*. However, their life cycles are not fully known, and it is impossible to determine the degree of relationship at the present time. The former genus was erected by Scherffel (**1926**) for two species, *M. cristata* and *M. fischeri*, which parasitize *Hyalotheca dubia* and *Zygogonium* sp. in Hungary. Subsequent studies by Sparrow (**1932**), Cejp (**1932**, **1933**), and Canter (**1949**) indicated a wider distribution of these species as well as the presence of other species in algae. Canter's excellent study in particular has broadened our concepts of the aquatic Synchytriaceae. She completed our knowledge of the life cycle of Scherffel's *M. fischeri* and described two new species, *M. intermedia* and *M. mirabilis* in England. Thus, *Micromycopsis* at present includes five species and one variety.

It differs from the aquatic (*Micromyces*) species of *Synchytrium* in that the prosorus develops an exit tube or canal of variable length through which its protoplast flows to the outside of the host cell and forms an epibiotic sporangial sorus. Occasionally, however, the latter may develop endobiotically without the formation of an exit canal as in the former *Micromyces* species. Also, it should be noted here that *S.* (*Micromyces*) *ovalis* rarely develops an exit canal as in *Micromycopsis* species, which apparently indicates a close relationship. Another difference noted occasionally by Scherffel in *M. cristata* and confirmed by Canter for *M. fischeri* is that the uniflagellate zoospores from the sporangia may exhibit only jerky or amoeboid movements after which they soon become quiescent and round up. Their protoplasm then undergoes cleavage into segments which develop into minute secondary uniflagellate zoospores and swim actively away. Accordingly, *Micromycopsis* develops primary and secondary sporangial phases and produces both primary and secondary zoospores as well.

Endodesmidium was created by Canter (**1949**) for an endobiotic parasite of *Netrium oblongum*, *Cylindrocystis crassa* and *C. brevissonii*. It produces a prosorus which germinates when mature and gives rise to a sessile endobiotic sorus. The latter develops two lateral exit papillae which may or may not pierce the host cell wall.

Its protoplasm cleaves into about fifty globular segments which ooze out singly in succession through the papillae, either into the external medium or into the cavity of the host cell. These bodies rarely develop a posterior flagellum but usually they are sluggish or remain quiescent, or undergo amoeboid movement. They are potential sporangia and soon become quiescent and round up. Subsequently, their content cleaves into 2 to 5 minutes posteriorly uniflagellate zoospores as noted occasionally in *Micromycopsis*. On one hand, *Endodesmidium* appears to have only one sporangial phase in the development of which the potential sporangia are discharged through exit papillae from the sorus and may behave for a short time as rudimentary primary zoospores. If, on the other hand, the discharged potential sporangia actually become motile and behave like zoospores, the entire sorus might be regarded as a primary sporangium.

Resting spores have not been observed in *Micromycopsis* and *Endodesmidium*. Therefore, it is not known whether they function directly as zoosporangia (*S. endobioticum*) or as prosori (*S. zygogonii*, etc.) in germination. Nevertheless, the development of exit canals by the prosorus (*Micromycopsis*) and papillae by the sorus (*Endodesmidium*) as well as the presence of primary and secondary zoospores are unknown in *Synchytrium*, and these are the principal differences which separate the genera at present. It is not altogether improbable that secondary zoospores may be found in species of *Synchytrium* also when they have been studied more carefully, and this is a developmental phase which should be searched for and investigated intensively.

In this connection it should be noted again that *Micromycopsis fischeri* occasionally forms an endobiotic sessile sorus without developing an exit canal. Such thalli are almost indistinguishable from those of the former *Micromyces* species. For this and other reasons Canter believes that ". . . it is clear that in the near future *Micromyces* and *Micromycopsis* may have to be merged into one genus." Rieth's (**1950B**) recent discovery that *S.* (*Micromyces*) *ovalis* rarely forms an exit canal lends support to Canter's view, or at least indicates that the two genera are closely related. However, so far as our knowledge goes, *Micromyces* usually has the same life

cycle as some species of *Synchytrium*, and these algal parasites may possibly represent a transition series from *Endodesmidium* and *Micromycopsis* to the terrestrial species of *Synchytrium*. Canter regards *Endodesmidum* as the most primitive genus of the Synchytriaceae. From this genus through *Micromycopsis fischeri, M. cristata* and *M. zygnaemicola* in succession the secondary sporangial phase is gradually suppressed until it is lacking in the aquatic (*Micromyces*) and terrestrial species of *Synchytrium*.

Classification of all *Synchytrium* species in subgenera is impossible at present because so few of them are fully known. Of the 158 or more species reported in the literature less than 25 are completely known and can be classified with certainty. Unfortunately, most studies on *Synchytrium* during the last three decades or more have been taxonomic and very little attention has been given to life cycles, developmental sequences and host range. As a result the majority of species are known only in their sorus, or prosorus, or resting spore stages. Other more recent studies (Cook, **1945–53**) have been concerned primarily with the type of galls produced on the host plants as a possible aid in classification. Unfortunately, many of the fungi were neglected so much in these studies that it is impossible in some cases to determine whether the investigator was describing prosori, or sori, or resting spores, and many of the new species created by Cook will have to be reinvestigated thoroughly before they can be properly classified. The same is true of the new species reported by Mhatre and Mundkur (**1945**), Patel, Kulkarni and Dhande (**1949**),[1] Padwick (**1945**), Lacy (**1950**), Ramakrishnan (**1950**) and Payak (**1951**).

The type of gall produced is helpful in distinguishing species, but it is not always definitely indicative of the type of life cycle and development. Furthermore, galls bearing sori or prosori may be quite different from those which bear the resting spores, *i.e. S. pulverum*. In most cases gall characteristics can be of only secondary value in taxonomy.

[1] The name *S. phaseoli* has already been given to a species by Weston (**1930**). Accordingly, their use of this binomial is invalid, and I am proposing the name **S. indicum** (Patel, Kulk. & Dh.) comb. nov. for their species.

As noted above, very few species are completely known as far as life cycle and development are concerned. Those which are fully known may be grouped into six subgenera as indicated in the key below.

KEY TO THE SUBGENERA OF SYNCHYTRIUM

I. Resting spores unknown. Mature thallus functioning directly as a summer sorus of thin-walled sporangia; sporangia delimited by cleavage within the soral wall, freed by its rupture, and appearing as powdery masses in open aecidium-like pustules.....Subgenus *Woroninella*

II. Resting spores known

A. Life cycle including summer sporangial sori and resting spores.

1. Mature thallus functioning directly as a sorus of thin-walled sporangia; sporangia delimited by cleavage within the sorus wall and freed by its rupture.

a. Resting spore functioning as a sporangium in germination and giving rise directly to zoospores.....Subgenus *Eusynchytrium*

b. Resting spore functioning as a prosorus in germination; content emerging to form a thin-walled sorus which cleaves into sporangia.....Subgenus *Exosynchytrium*

2. Mature thallus functioning as a prosorus; content emerging from initial cell to form a thin-walled sorus which cleaves into sporangia.

a. Resting spore functioning as a sporangium in germination and giving rise directly to zoospores.....Subgenus *Mesochytrium*

b. Resting spore functioning as a prosorus in germination; content emerging to form a thin-walled sorus which cleaves into sporangia.....Subgenus *Microsynchytrium*

B. Life cycle including only resting spores; summer sporangial sori unknown.

1. Resting spore functioning as a prosorus in germination.

a. Protoplast emerging to form a thin-walled sorus which cleaves into sporangia.....Subgenus *Pycnochytrium*

2. Resting spore functioning as a sorus in germination.

a. Protoplast not emerging; cleaving directly into several sporangia which dehisce irregularly with the rupture of the resting spore wall.....Subgenus *Endosynchytrium*

In 1933 DuPlessis added the subgenus *Endochytrium* as a provision for his *S. cotulae,* but inasmuch as this name had already been used by Sparrow (**1933**) for another chytrid genus it is invalid. Accordingly, the author (**1941**) proposed the name *Endosynchytrium* for this subgenus.

This key is an extension and modification of the one proposed by Fitzpatrick (**1930**), and is based on the presence or absence of summer sporangial sori, or prosori and resting spores, the type of resting spore germination, as well as on the development of the

mature vegetative thallus into a sorus or its function as a prosorus. The general noncommittal term resting spore is used for the relatively dormant, thick-walled stage, regardless of whether it functions as a prosorus, or sorus, or a sporangium in germination, or whether or not it is formed as the result of fusion of motile isogametes. Also, no distinction is made between sporangia and gametangia or between zoospores and gametes because they are morphologically and structurally alike so far as is known at present. Sexual reproduction has been reported in only *S. endobioticum, S. fulgens,* and *S. longispinosus,* and it is difficult to incorporate distinctions on this basis at present in a general key to all species of *Synchytrium.* Further division of the subgenera into groups such as *Leucochytrium* and *Chrysochytrium* on the basis of color is not as significant taxonomically as it was believed to be. Color of the thallus is not a reliable criterion because it varies markedly with age. Thalli may be almost hyaline or lemon-yellow when young and gradually become dark yellow to deep orange with maturity. Obviously, any key is only provisional, and the proposed one will doubtless have to be modified markedly as old species become better known and new ones are added to the genus.

On the basis of present-day knowledge the following reported species may be assigned provisionally to the various subgenera listed above.

WORONINELLA: *S. accidioides, S. minutum, S. citrinum, S. psophocarpi, S. vulcanicum, S. dolichi, S. atylosiae, S. vignicola, S. aequatoriensis, S. phaseoli* and *S. crotulariae.*

EUSYNCHYTRIUM: *S. taraxaci.* In *S. papillatum, S. sanguineum, S. geranii, S. andinum, S. amsinckae, S. trichophilum, S. uliginicola, S. cellulare, S. australe, S. valerianellae* and *S. linderniae* both sporangial sori and resting spores have been reported, but germination of the latter has not been observed. Therefore, it is not certain that they belong in *Eusynchytrium.*

EXOSYNCHYTRIUM: *S. fulgens.*

MESOCHYTRIUM: *S. endobioticum.* Usually, *S. succisae* and *S. stellariae* are included in this subgenus, but whether or not they belong here will not be certain until germination of their resting spores has been observed.

MICROSYNCHYTRIUM: *S. zygogonii, S. longispinosus,* and *S. laevis.* Whether or not *S. spirogyrae, S. petersenii* and *S. ovalis* belong in this subgenus will not be certain until germination of their resting spores has been observed.

PYCNOCHYTRIUM: *S. myosotidis, S. lateum, S. punctatum, S. punctum, S. pilificum, S. potentillae, S. fuscus, S. mercurialis, S. globosum, S. rubrocinctum, S. pyriforme, S. musicola* (?), *S. alpinum, S. scirpi, S. aureum, S. ulmariae, S. auranticum, S. anemones, S. anomalum, S. alpinum,* etc.

ENDOSYNCHYTRIUM: *S. cotulae.*

The remaining 80 or more species of *Synchytrium* are too poorly known to be classified with any degree of certainty in the above subgenera. It is obvious that an immense amount of research remains to be done on *Synchytrium,* and it is hoped that present and future investigators will study the individual species more intensively instead of making new species on incomplete and fragmentary evidence.

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SOME NEW SPECIES OF SYNCHYTRIUM FROM BANARAS

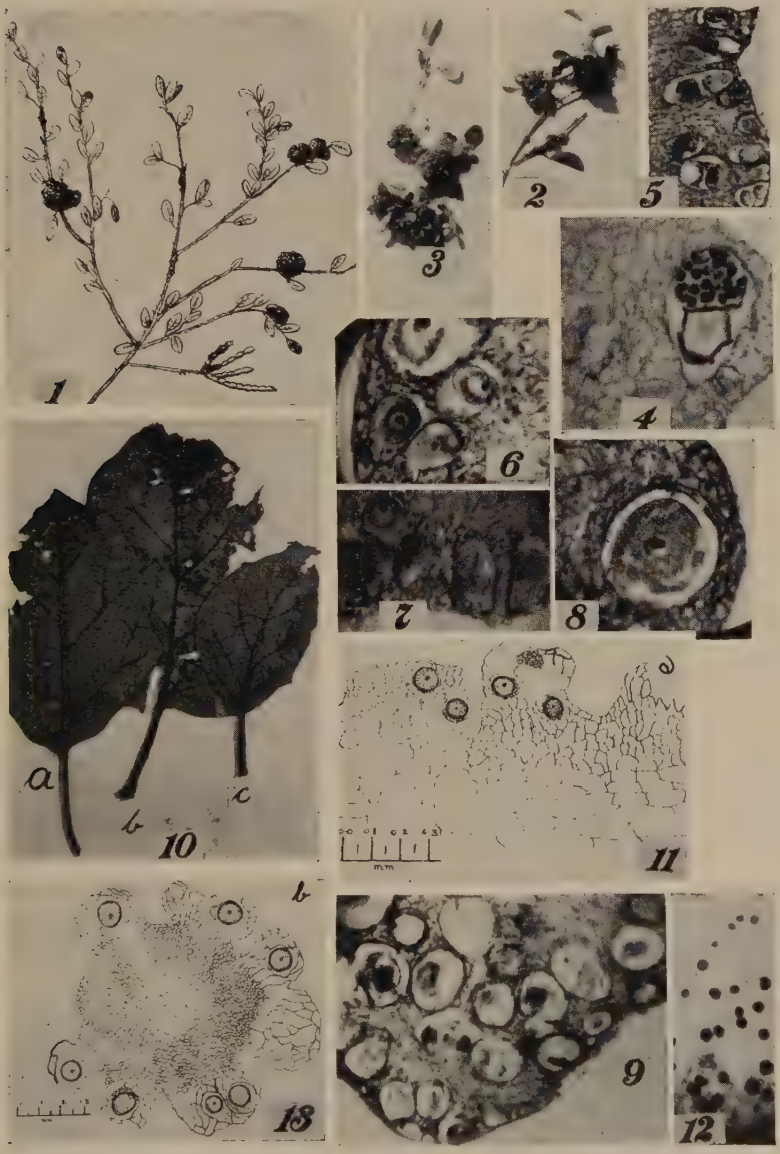
B. T. Lingappa

(with 13 figures)

A SYNCHYTRIUM CAUSING A WART DISEASE OF ALYSICARPUS MONILIFER DC.

This interesting new species was found by the author at Banaras, India. The host plant is a moderately spreading perennial weed very common in the lawns and along the road sides. All aerial parts die out during summer but make a fresh growth with the coming of rains in September. All the aerial parts, leaves, petioles, stems, fruits, and especially the axillary and spical buds become infected (Fig. 1). The infected parts produce generations of summer sori, and this leads to extensive proliferation of the tissue and results in the formation of warts. These warts are conspicuous at the close of the season (Figs. 2, 3). They are light green to yellow and later become brown. They show different stages of prosori in the earlier stages and the hypnospores dominate as the season advances. Sometimes, fast developing tender shoots become severely infected and develop into a whorl of prominent wart-aggregates at the crown. At the close of the season the warts are full of hypnospores which dry up, crumble and are mixed with the soil. During the next rainy season the new branches develop and come in close contact with the soil, due to their creeping habit. The tender parts are thus easily accessible to the zoospores and the disease is carried on. Wet rot of the warts does not occur.

The galls on the leaves are minute, spherical or cupulate and yellow. Galls containing prosori rupture, become cupulate and release zoosporangia. Leaves suffering from the production of many generations of summer sori become much distorted and yellow. Galls produced later contain hypnospores, are simple, much smaller and produce less distortion.



FIGS. 1–13.

The warts produced in this case are very similar to those of the potato wart in morphology and development. All the warts are aerial. The sequence of development of this fungus is also similar to *S. endobioticum* (Schilb.) Perc. The zoospores gain entrance into the epidermal cells of the host. Almost every other epidermal cell may be infected (FIG. 5). Multiple infections are very common (FIGS. 5, 7). More than three fungus bodies were frequently seen developing in a single cell. Soon after entry they become spherical but may become elongated when more than two are growing in a cell. The zoospores enter the host cell without leaving any path of entry. The nuclear structure attains its maximum size very early (FIG. 6). The nucleolus lies in the center of the nuclear vacuole and is deeply stained. The fungus at maturity has a thin yellow exospore and much thinner hyaline endospore. It develops into a prosorus. The contents of the prosorus flow into an extruded thin vesicle. The nucleus also passes into it and undergoes numerous divisions. Cleavage planes appear, breaking the whole mass into uninucleate polyhedral pieces which develop into sporangia. The vesicle is extruded, not through a small slit in the exospore as reported in the formation of prosori in other *Synchytrium* species, but is formed by an elongated rupture of the exospore (FIG. 4). The zoosporangia are pyriform or oval, yellow, and undergo much distension causing the rupture of the galls. The exospore of the prosorus, which is much shrivelled, thin, dirty yellow, and boat-shaped, may remain behind the cupulate galls.

Later in the season, the warts are full of thick-walled hypnospores. It is only in the cells containing the mature hypnospores that the cell contents precipitate as a brown mass. But the contents of the host cells in which prosori are developing are not so affected even up to the time of their dehiscence. The host nucleus, however, disintegrates very early in both cases. Though multiple infections were so common, the development of more than one hypnospore is rare and invariably only one prosorus develops in a single host cell. Differences in the age of the developing fungus bodies were common (FIG. 7). It may be possible that in cases of multiple infections only one of them will ultimately develop while others become stunted and disappear later.

Synchytrium cookii sp. nov.

Gallae minutae, luteae, sphericae, in utraque pagina foliorum. Gallae cupulatae plus aggregatae quam gallae sphericae, 0.2–0.3 mm. diam., distortionem partium infectarum causantes. Alabastra axillaria affectata atque partes juniores culmorum evolvuntur in excrescentias verruculosas 2 cm. diam., vel largiores, luteas, postea brunneas. Cellulae epidermales infectae. Infectio multiplex communis est. Ut plurimum unus prosorus vel una hypnospora in una cellula evolvitur. Prosorus exosporio tenui, luteo et endosporio hyalino ornatus. Sporangia 50–70, spherica vel pyriformia, lutea, in vesiculo extruso delimitata, 20–22–26 μ diam. Hypnosporae plurimae, singulae in cellulis epidermalibus, parietibus crassis praeditae, dense luteae, sphericae vel ellipsoideae, contentis luteis granularibus, 50–85–120 μ diam. Exosporium 5 μ crassum.

Typus afficiens alabastra axillaria, folia atque culmos *Alysicarpi moniliferi* DC. a B. T. Lingappa inventus, in loco Banaras, Indiae, die 12 septembris 1950.

Galls minute, yellow, spherical, on both surfaces of the leaves, also on axillary buds and stems. Cupulate galls more crowded than the spherical galls, measuring 0.2 to 0.3 mm. in diameter, causing distortions of the affected parts. Affected axillary buds and tender parts of the stem developing conspicuous warty outgrowths, measuring 2 centimeters or more in diameter, yellow, becoming brown. Epidermal cells infected. Multiple infection common. Commonly one prosorus or a hypnospore develops in a single cell. Prosorus with a thin yellow exospore and a hyaline endospore. Sporangia 50 to 70 in number, spherical to pyriform, yellow, delimited inside the extruded vesicle, 20–26 μ (av. 22 μ) in diameter. Hypnospores numerous, one inside an epidermal cell, thick-walled, deep yellow, spherical or ellipsoid, with yellow granular contents, measuring 50–120 μ (av. 85 μ) in diameter. Exospore 5 μ thick.

A SYNCHYTRIUM ON BRINJAL PLANTS (SOLANUM MELONGENA)

A species of *Synchytrium* was found attacking Brinjal plants near the Hindu University in 1950 and again in September 1951. The fungus attacks leaves and other tender parts. This is followed by the formation of simple or aggregated galls on both surfaces (FIG. 10) which are very abundant near the tips and along veins. Infections are also severe on petioles and stems, which become encrusted and irregularly thickened. They also become crustaceous on the midribs. They are brown and thickened. The distortions of the leaves are not severe, but the leaves are thicker than usual.

The galls are spherical at first but become cupulate with the dehiscence of the prosori. Such erumpent galls may coalesce. The hypnospores are produced in much smaller galls. The galls are yellow, becoming dirty brown. The cupulate galls are 0.4–1 mm. in diameter, while the galls containing the hypnospores are up to 0.2 mm. in diameter. Each gall may contain three or more hypnospores but only one hypnospore in a cell (FIG. 11). The summer spores become prosori. The sporangia are 30 to 40 in number, yellow, spherical at time of dehiscence and become pyriform or beaked later. Zoosporangia at time of release from vesicle measure 16–18 μ in diameter. Hypnospores spherical or oval, dark brown, with 6–9 μ thick brown exospore. The disintegrated host cell contents form a third wall over the hypnospore. Infections spread from nursery beds and the seedlings raised during heavy rains are attacked most severely. The disease under such conditions spreads rapidly to the growing stem apex and the seedlings so infected are useless for transplanting. Other records on Solanaceous hosts differ much from this fungus which is a member of the subgenus *Mesochytrium*.

Synchytrium akshaiberi sp. nov.

Gallae minutae, sphericae, leves, nitentes, luteae vel brunneae, per foliorum paginas dispersae, 90–250 μ diam. Gallae cupulatae largiores, haud altae, ovatae, crustaceae, 0.8–1.5 mm. diam., plus aggregatae in foliorum petiolis, culmis atque nervis mediis incrassationes irregulares causantes, luteae, postea brunneae. Prosori lutei, parietibus crassis ornati. Sporangia 30–40, ovalia, lutea, 16–18 μ diam., delimitata in vesiculo extruso. Hypnospora una vel plures in singulis gallis, singulae in cellulis plantae hospitis, dense brunneae, parietibus crassis ornatae, sphericae, raro oblongae, 48-92–130 μ diam., contentis luteis granularibus. Exosporium 6–9 μ crassum.

Typus in foliis, petiolis atque culmis *Solani melongenae* L., in loco Banaras, India, die 21 septembris, 1950, a B. T. Lingappa inventus.

Galls minute, spherical, smooth, glistening, yellow to brown, studded over the leaf surfaces, measuring 90–250 μ in diameter. The cupulate galls are bigger, shallow, oval, crustaceous, measuring 0.8–1.5 mm. in diameter, crowded on the petioles, stem and midribs causing irregular thickenings, yellow, later turning brown. Yellow, thin-walled prosori formed. Sporangia 30–40 in number, oval, yellow, measuring 16–18 μ in diameter, delimited within an extruded vesicle. Hypnospores one or more in a gall, one inside

a single host cell, dark brown, thick-walled, spherical, rarely oblong, measuring 48–130 μ (av. 92 μ) in diameter, with yellow granular contents. Exospore 6–9 μ thick.

A SYNCHYTRIUM ON MELILOTUS

The fungus attacks *Melilotus indica,* a very common weed in the rainy season. The disease appears on all parts of the plants for a very short period and causes extensive defoliation. The stems and branches often show crowded patches of spherical yellow galls which turn dark brown later. The infected leaves are distorted, turn yellow and are soon shed. The life-history of the fungus is very short. After a long period of exposure to rains the dormant hypnospores in the soil release zoospores which invade the epidermal cells of the host and develop directly into big yellowish brown hypnospores. The galls are simple, yellow, becoming brown; each of them contains one to four hypnospores. But only one hypnospore occurs in a single host cell. At the maturity of the hypnospores, disintegrated, brown contents of the host cells are deposited on the exospores as a third layer. This species is a *Chrysochytrium* of the subgenus *Pycnochytrium.* It differs markedly from the two above-mentioned species of the subgenus *Mesochytrium* in having a very short life-cycle. The disease appears quite late in the season; possibly the hypnospores have a prolonged period of dormancy and therefore germinate after a prolonged period of exposure to rains. The fungus is insured against the possibility of complete destruction by the rapid shedding of the affected leaves soon after the hypnospores have matured.

Synchytrium meliloti sp. nov.

Gallae prominentes, sphericae, leves, pallide luteae, 0.2–0.4 mm. diam., in petiolis, culmorum partibus atque in utraque pagina foliorum aggregatae. Folia affectata distorquentur, aliae partes tenuiter incrassantur, brunneae. Sporangia nulla observata. Hypnosporae largae, sphericae, pallide brunneae, una tantum vel plures tribus in una galla, una in cellula epidermali, 100–120–165 μ diam., contentis luteis, minute granularibus.

Typus in foliis atque culmis *Meliloti indicae* All. in loco Banaras, India, die 25 septembris 1951, a B. T. Lingappa inventus.

Galls prominent, spherical, smooth, light yellow, measuring 0.2 to 0.4 mm. in diameter, crowded on petioles and stem parts and on

both surfaces of the leaves. Infected leaves distorted; other parts slightly thickened and turning brown. No sporangial formation observed. Hypnospores large, spherical, light brown, one or rarely more than three in a gall; one in an epidermal cell; 100–165 μ (av. 120 μ) in diameter, with finely granular yellow contents.

In proposing the above three species as new, the author has closely followed the latest trend of work in this genus (3). Field observations and frequent examinations of fresh collections have facilitated an understanding of the true nature of these species.

The type specimens of the above species will be deposited at the Herb. Crypt. Orient. New Delhi. The author is highly thankful to his professor Dr. Akshaiber Lal for the encouragement and facilities; to Rev. Father Dr. H. Santapau for translating the diagnoses into Latin, and to the officers of the Herbarium at I.A.R.I. New Delhi for providing facilities to examine the collections available there. Thanks are due to Mr. D. L. Bohra, college artist, for photomicrographs. The author is much indebted to Drs. M. T. Cook, B. B. Mundkur and Robert Page, for reprints. Dr. M. T. Cook has kindly gone through the paper and made valuable corrections for which the author is much indebted.

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EXPLANATION OF FIGURES

FIGS. 1–9. *Synchytrium cookii.* 1. A portion of *Alysicarpus monilifer* showing symptoms of infection. 2. Warts of axillary buds and stem. 3. Warts of the stem apex. 4. Zoosporangia formed in the extruded vesicle. Empty case of the prosorus still intact with the vesicle. Vesicle extruded through the wide opening. 5. Severe infection of the epidermal cells, showing multiple infection. 6. Prominent nucleolus and nucleate vacuole in all the

three very young stages of the fungus bodies. 7. Multiple infection showing differences in age of the developing fungus bodies. 8. Young developing prosorus, highly enlarged. 9. Showing the distribution of hypnospores within the wart tissue.

FIGS. 10—11. *Synchytrium aksheiberi.* 10. Brinjal leaves showing distribution of galls. Characteristic symptom on petioles and midribs as compared to petiole of 'a.' 11. Section through an affected leaf showing the galls, cupulate gall hypnospores and zoosporangia in extruded vesicle. Portion of cupulate gall.

FIGS. 12–13. *Synchytrium meliloti.* 12. Enlarged view of the hypnospores in the galls and their distribution on the leaf surface. × 24. 13. Section through affected stem showing nature and distribution of galls and hypnospores.

THE NEW RULES OF TYPIFICATION AS THEY AFFECT SARCOSCYPHA AND VELUTARIA

Richard P. Korf

The new rules covering the selection of a type (Lanjouw 1951) will serve to answer many perplexing situations in botanical nomenclature. Two nomenclatural problems in the Discomycetes have puzzled me for some time. The rules, as written, provide a not too laborious solution to these problems. The result of one of these is encouraging, in that it allows the use of names long current in the literature and well-known even to those with but a cursory interest in the cup-fungi. The result of the other, however, is displeasing in that it means using a well-known name in a sense in which the name has rarely been employed, and may leave another group of species without an available generic name.

The two problems outlined below have certain points in common: the genera were erected originally with two species each, and in neither case was a type designated. Neither the new Art. 19 nor Recommendation V (4e) will apply, as it is not possible to say in either case that one of the species fits the generic diagnosis better than the other. Article 18, Note 3 seems to decide the issue in both cases:

"If no holotype has been indicated by the author who described a taxon, or when the holotype is lost or destroyed, a substitute for it must be chosen. The author who makes this choice must be followed unless it can be proved that the choice was not made in accordance with Art. 19. The substitute may be either a *lectotype* or a *neotype*. A lectotype always takes precedence over a neotype.

"A *lectotype* is a specimen or other element selected from the *original material* to serve as nomenclatural type when the holotype was not designated at the time of publication or so long as it is missing.

"When two or more specimens have been designated as types by the author of a name (i.e., male and female, flowering and fruiting, etc.) one of them must be chosen as *lectotype*.

"A *neotype* is a specimen selected to serve as nomenclatural type so long as all of the material on which the name of the taxon was based is missing."

PLECTANIA, SARCOSCYPHA, AND RHIZOPODELLA

The common scarlet-cup of the early spring has passed for many years under the name *Sarcoscypha coccinea.* This nomenclature was overturned when Seaver (**1928**) revived the name *Plectania coccinea* for the fungus. Recent authors have followed Seaver, notably Kanouse (**1948**) and Nannfeldt (**1949**). The discovery of different types of spore germination in this species and in what is presumably *S. coccinea* var. *jurana* Boud. (Rosinski **1953**) led to an investigation of the correct generic name to apply to the scarlet-cups.

Plectania was erected by Fuckel in 1870 to include two species, *P. coccinea* (Scop. ex Fr.) Fckl. and *P. melastoma* (Sow. ex Fr.) Fckl. One of these species must be designated the lectotype.

Boudier (**1885**) founded two genera containing these species: *Sarcoscypha* (Fr.) Boud., based on *Peziza coccinea* Scop. ex Fr. and *P. occidentalis* Schw.,[1] and *Rhizopodella* (Cooke) Boud., with *Peziza melastoma* Sow. ex Fr. designated the type. Depending upon the choice of a lectotype for *Plectania* Fuckel, one or the other of Boudier's genera must be considered a synonym of *Plectania.*

Saccardo (**1889**) was apparently the first author to divide the genus *Plectania* into two. Inasmuch as the genus originally contained only two species, this division automatically selected a lectotype. He recognized two genera for the species involved: *Plectania* Fckl., including *P. melastoma* and a number of additional species, and *Sarcoscypha* (Fries) Boudier (as "Fries"), embracing *S. coccinea* and certain other species.

When the new rules of typification are applied, which consider the principle of priority, it is clear that it is necessary to return to the generic name *Sarcoscypha* for the scarlet-cups, instead of using

[1] The latter species is still considered congeneric with *P. coccinea* in all recent works known to me. It was not treated by Fries, however, and is therefore not eligible as the type of the Fries subgenus. The only other species known to Fries and included by Boudier in the genus is *P. coccinea,* which must be considered as the lectotype of the genus.

Plectania as some recent authors have done. Further, the correct generic name for *Peziza melastoma* is *Plectania,* a name by which it has long been known, and not *Rhizopodella,* as used by Kanouse (**1947, 1948**), Nannfeldt (**1949**) and Korf (**1949**). The synonymy and typification of the genera is therefore as follows:

PLECTANIA Fuckel, Symb. myc. 323. [1870.], *emend.* Sacc. Syll. Fung. **8**: 163. 1889.

SYNONYMS: *Peziza* ser. *Lachnea* subgen. *Rhizopodella* Cooke, Mycogr. **1**: 260. 1879. [Lectotype: *Peziza melastoma* Sow. ex Fr., designated by Boudier (**1885**).]

Peziza subgen. *Plectania* (Fckl.) Sacc. Bot. Centralbl. **18**: 215. 1884. (Holotype: *Peziza melastoma* Sow. ex Fr.)

Rhizopodella (Cooke) Boud. Bull. Soc. Myc. France **1**: 103. 1885. [Holotype: *Peziza melastoma* Sow. ex Fr. (*Rhizopodella melastoma* (Sow. ex Fr.) Richon, Catalogue raisonné des Champ. 191. 1889.)]

TYPE SPECIES: *Peziza melastoma* Sow. ex Fr. (*Plectania melastoma* (Sow. ex Fr.) Fckl. Symb. myc. 324. [1870.]), effectively selected by Saccardo (**1884, 1889**).

SARCOSCYPHA (Fries) Boudier, Bull. Soc. Myc. France **1**: 103. 1885.

SYNONYM: *Peziza* ser. *Lachnea* trib. *Sarcoscyphae* Fries, Syst. myc. **2**: 78. 1822. [Lectotype: *Peziza coccinea* Scop. ex Fr. Syst. myc. **2**: 79. 1822, effectively selected by Boudier (**1885**).]

TYPE SPECIES: *Peziza coccinea* Scop. ex Fr. (*Sarcoscypha coccinea* (Scop. ex Fr.) Lambotte, Fl. myc. belg., suppl. 1, 302. 1887) effectively selected by Boudier (**1885**).

Note that the combination *S. coccinea* is usually erroneously attributed to Saccardo rather than to Lambotte.

VELUTARIA, TAPESINA, ETC.

The genus *Velutaria* was erected by Fuckel in 1870, and included two species, *V. rufo-olivacea* (Alb. & Schw. ex Fr.) Fckl. and *V. grisco-vitellina* (Fckl.) Fckl. In 1887,[2] the genus *Tapesina* was founded by Lambotte, and likewise included two species, *T. ruborum* (Cooke & Phil.) Lambotte and *T. retincola* (Rabenh.) Lambotte. The latter species is today considered a true *Tapesia.* The former

[2] In my monograph of the Arachnopezizeae (Korf **1951**), I gave the date 1888 for Lambotte's paper. This appears to be in error, as the separates of this work were dated (and presumably issued in) 1887, while the volume of the Mém. Soc. Roy. Sc., Liège, in which the work was published, appeared in 1888. The citations in my paper (*loc. cit.*, pp. 150, 151) should therefore read: Fl. myc. belg., suppl. 1 (same pages), 1887.

is a synonym of *Peziza griseo-vitellina* Fckl., and treated as the type of *Tapesina* under the name *T. griseo-vitellina* (Fckl.) Höhnel. It is so used by von Höhnel (1923), Nannfeldt (1932), Kanouse (1947), Dennis (1949), Gremmen (1951) and Korf (1951).

The generic name *Velutaria* has come to be applied to *V. rufo-olivacea* and its allies. It is treated thus by Rehm (1892), Lindau (1897), Nannfeldt (1932), Kanouse (1947), Petrak (1949), Gremmen (1951), Groves (1951) and Seaver (1951). Such unanimity of opinion is rarely reached in discomycete nomenclature. A strict application of the new rules of typification will, however, completely disrupt this harmony, upsetting the very stability of nomenclature which the rules are intended to supply.

The case is not unlike that of *Plectania*. Again Saccardo was apparently the first to divide the genus. In 1884 he emended the genus, designating *V. griseo-vitellina* the type. He followed this in 1889 by leaving *V. griseo-vitellina* in the genus (adding two further species), and relegated *V. rufo-olivacea* to the genus *Lachnella* "Fries." He unquestionably selected a lectotype by this division.

If what we had agreed to call *Tapesina* need now be termed *Velutaria*, it is necessary to find a name for the species we were calling *Velutaria*. Unfortunately, this problem is by no means a simple one, and it may well be that no generic name is available for them. In his historical account of the genus *Velutaria*, Petrak (1949) makes no mention of *V. griseo-vitellina*, but rather merely states that *V. rufo-olivacea* is the type species. *Phaeangium rubi* (Bäuml.) Sacc. is stated by Petrak to be a synonym of *V. rufo-olivacea*, and to be the "type" of *Phaeangium* (Sacc.) Sacc. & Syd. 1902. It is similarly designated the type by Nannfeldt (1932). (The type, however, should be one of the original species included by Saccardo in *Cenangium* subgen. *Phaeangium*.) In any case, this generic name is a later homonym of *Phaeangium* Patouillard 1884. *Schweinitzia* Massee is also unavailable, as it is a later homonym of *Schweinitzia* Greville 1823.

The only generic name which might be legitimate appears to be *Phaeangella* (Sacc.) Sacc. & Sacc. 1906. Petrak (1949) terms *P. aceris* (Hazsl.) Sacc. & Sacc. the "type" of the genus, again pre-

sumably because it was the first species listed in the genus (and likewise the first species listed in *Cenangella* subgen. *Phacangella* Sacc. 1889). Petrak suspects that *P. aceris* is also a synonym of *V. rufo-olivacea*. Should this lectotype prove to be the first designated and also to be a synonym of *V. rufo-olivacea*, a generic name would become available for that species and its allies.

A strict application of the rules would then result in the following (unfortunate) synonymy and typification:

VELUTARIA Fuckel, Symb. myc. 300. [1870.], *emend.* Sacc. Bot. Centralbl. **18**: 219. 1884.

SYNONYM: *Tapesina* Lambotte, Fl. myc. belg., suppl. 1, 305. 1887, *emend.* Höhnel, Sitz.-ber. Akad. Wiss. Wien, Math.-nat. Kl., I, **132**: 110. 1923. [Lectotype: *Peziza ruborum* Cooke & Phil. (*Tapesina grisco-vitellina* (Fckl.) Höhn. Ber. Deutsch. Bot. Ges. **37**: 108. 1919.)]

TYPE SPECIES: *Peziza grisco-vitellina* Fckl. (*Velutaria grisco-vitellina* (Fckl.) Fckl. Symb. myc. 300. [1870.]), designated by Saccardo (**1884**).

? PHAEANGELLA (Sacc.) Sacc. & Sacc. Syll. Fung. **18**: 128. 1906, *emend.* Petrak, Sydowia **3**: 199. 1949.

SYNONYMS: *Cenangella* subgen. *Phacangella* Sacc. Syll. Fung. **8**: 587. 1889. [Lectotype: *C. aceris* (Hazsl.) Sacc., selected by Petrak (**1949**).]

Cenangium subgen. *Phacangium* Sacc. Syll. Fung. **8**: 570. 1889. (Lectotype: ?)

Schweinitzia Massee, Brit. fungus-fl. **4**: 134. 1895. (Lectotype: ?) Not *Schweinitzia* Greville, Edinburgh Phil. Jour. **8**: 258. 1823.

Phacangium (Sacc.) Sacc. & Syd. Syll. Fung. **16**: 764. 1902. (Lectotype: ?) Not *Phacangium* Pat. Jour. de Bot., i, **8**: 155. 1894.

TYPE SPECIES: *Cenangium aceris* Hazsl. (a new combination in *Phacangella* would be necessary for *rufo-olivacea*), selected by Petrak (**1949**).

Both *Velutaria* and *Tapesina* as they are used in the literature are small genera, and it is questionable whether conservation should be utilized for such cases. When the rules result in the chaos exhibited above, however, it is necessary to consider whether the rules are wisely drawn. Many of the rules which have been proposed in the past have emphasized that usage is often a better guide than strict priority. Since the rules as now written include the priority concept, it would be necessary to conserve *Velutaria* in its current sense over *Velutaria* as emended by Saccardo, to avoid application of the rules. The case for conservation of small genera is tenuous, at best. Unless the rules of typification are revised, however, one must either conserve some such genera or submit to an upheaval of the nomenclature of such groups. I would personally prefer to see the rules revised to take into account predominant

usage, as in the case of *Velutaria*. If this is not feasible, conservation must be recommended.

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TWO TYPES OF SPORE GERMINATION IN SARCOSCYPHA COCCINEA (SCOP. EX FR.) LAMBOTTE

MARTIN A. ROSINSKI

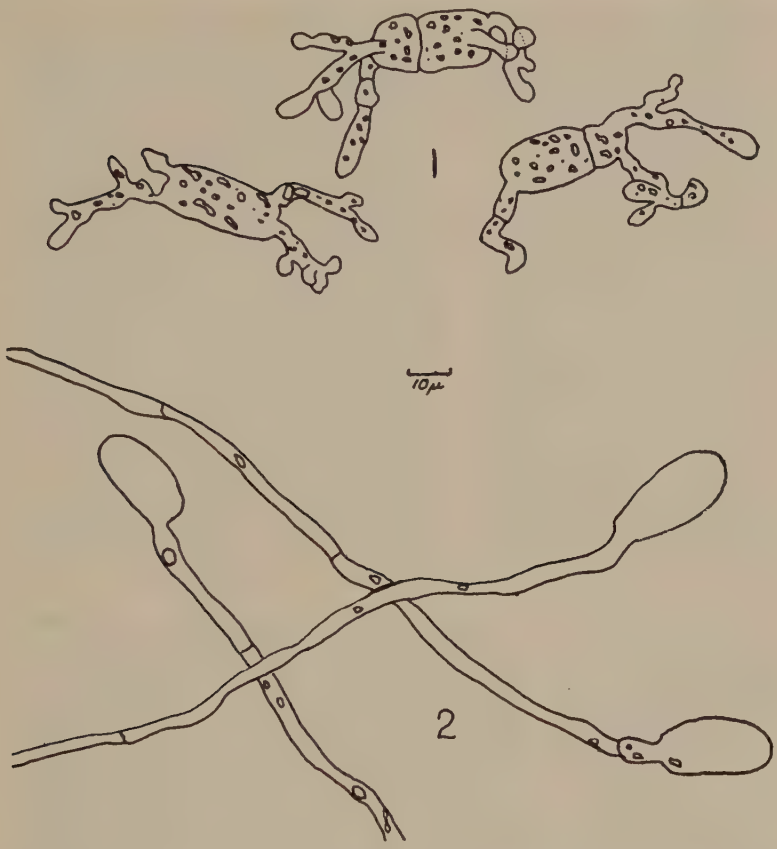
(WITH 6 FIGURES)

On April 19th, 1952, two collections of *Sarcoscypha coccinea*[1] were brought into the mycology laboratories at Cornell University. The collections were made independently from different local areas around Ithaca, New York. When these collections were placed side by side, it was obvious that there was a distinct color difference between them. In one collection, the hymenium was slightly on the orange side of scarlet (Séguy 167), while in the other collection the hymenium was a shade or two darker than scarlet (Séguy 61).

A variety, *S. coccinea* var. *jurana,* was described by Boudier (1903). He (1906–1907) later illustrated *S. coccinea* and *S. coccinea* var. *jurana,* showing a difference in the color of the hymenium and in the shape and size of the ascospores. In variety *jurana* he shows the hymenium with a brighter, more orange-red color, and the ascospores with flattened or blunt ends. In fact, in some cases the spores appear not only flattened, but indented at the ends. Ascospores from our collections which had been shot onto clean slides and onto agar showed a striking difference in morphology, strongly suggestive of the two forms illustrated by Boudier (FIGS. 3 and 5). However, the color of the hymenium in relation to the shape of the ascospores in our collections was the exact reverse of that indicated by Boudier. Perhaps color varies not with the variety but with the individual collection.

Spore measurements taken from the two collections (50 spores each) were as follows: 6.6–10.6 × 20–29 μ from the collection with blunt spores (presumably var. *jurana*), and 8–12 × 20–34.5 μ for the collection with tapered spores. Le Gal (1941) has shown that

[1] The valid generic name for the scarlet-cups is *Sarcoscypha*, as pointed out by Korf (1953).



FIGS. 1–2. Spore germination in *Sarcoscypha coccinea* on water agar after 4 days. 1. *S. coccinea* var. *jurana*. 2. *S. coccinea* var. *coccinea*. Camera lucida drawings, at × 740, reduced in reproduction to × 500.

spore measurements vary considerably in this species, and the above figures are probably not significant. She also points out that some blunt spores may be found in variety *coccinea*[2] and that some tapered spores may be found in variety *jurana*. She concludes that the difference is not sufficiently constant to maintain a distinction. It is possible that the two types of spore germination to be de-

[2] According to the new rule established at the Stockholm meetings in 1950 (Lanjouw **1951**), the establishment of *S. coccinea* var. *jurana* Boud. automatically erected another variety, *S. coccinea* var. *coccinea*, which is to be cited without an author's name.

FIGS. 3-4. *S. coccinea* var. *jurana*. 3. Spores on water agar after 24 hrs. 4. After 4 days.

FIGS. 5–6. *S. coccinea* var. *coccinea*. 5. Spores on water agar after 24 hrs. 6. After 4 days. All photomicrographs reproduced at × 500.

scribed may lend support to Boudier's conclusion that there are two taxons involved.

To observe spore germination, portions of apothecia were inverted over petri dishes of water agar and of potato-dextrose agar.

The apothecia were allowed to shoot their spores onto the agar for a period of 24 hours. Ascospores were observed by cutting out blocks of the agar from areas where it could be seen that spore discharge had occurred. These blocks were placed on clean slides, and slide cultures were made according to the technique of Riddell (1950). Subsequent observations were made at 24 hour intervals.

Spores from both collections were observed to germinate on water agar within 48 hours. Little or no germination on potato-dextrose was observed. In the case of the collection with blunt spores, which we are calling *S. coccinea* (Scop. ex Fr.) Lambotte var. *jurana* Boud. (CUP *38758*), germination on water agar was observed to occur in the same fashion as that noted by Alexopoulos and Butler (1949). After swelling of the spores, from one to four short germ tubes were produced which soon gave rise to small buds or "conidia" as they were termed by the workers mentioned above. Camera lucida drawings (FIG. 1) and a photograph (FIG. 4) show this mode of germination. Following germination, the ascospore itself may become one or more septate.

On the same medium, a strikingly different spore germination was noted in the other collection, which we are calling *S. coccinea* (Scop. ex Fr.) Lambotte var. *coccinea* (CUP *38757*). After swelling of the ascospores, in most cases only one germ tube was produced from a single spore, and never more than two. Further, the germ tubes, instead of producing "conidia," merely increased in length, became multiseptate, and ultimately started to branch and form a limited mycelium. Camera lucida drawings (FIG. 2) and a photograph (FIG. 6) show this type of germination.

The significance of these results is not, as yet, known. They are results from only two isolated collections. Further work should prove whether or not the two types of germination which we observed are a fundamental difference between the two varieties.

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NOTES AND BRIEF ARTICLES

Closure for Culture Bottle

Aerobic micro-organisms that grow rapidly in surface culture often need a larger opening in the culture vessel, in proportion to the growth surface area, than micro-organisms that have a slow rate of growth. It is, therefore, frequently necessary to grow micro-organisms in wide-mouth bottles rather than narrow-mouth bottles to obtain greater interchange of air. A cotton plug could be used as a closure for the wide-mouth bottle, but it has well-known disadvantages. We have devised the bottle cap assembly shown in Fig. 1 as a substitute for the cotton plug. This assembly is made from a standard molded plastic cap, filter paper, a rubber band, and a prepared glass sleeve. The top of the plastic cap is cut out, so that the diameter of the opening is approximately equal to the diameter of the bottle mouth. Sufficient edge is left on the cap to give a good seal with the glass edge of the bottle. A snug fitting flat rubber band is placed on the outside of the cap to prevent the glass sleeve from slipping, and also act as a cushion to close crevices in the folded edge of the filter paper. The cap is screwed on the bottle and covered with filter paper and the glass sleeve is then placed over it.

For our assembly, Schleicher and Schuell [1] sharkskin filter paper was used. It has high wet strength, resistance to repeated steam sterilization and good air permeability. The number of filter papers used should be sufficient to give a tight fit between the glass sleeve and the cap; a minimum of three pieces is recommended. As many as twelve pieces can be used without significant effect on the growth of micro-organisms. It may be necessary to add more filter paper to some assemblies after repeated use to maintain a tight fit between the glass sleeve and the cap.

The glass sleeve is made long enough to reach the shoulder of

[1] Mention of brand names does not constitute endorsement or recommendation by the Department of Agriculture over similar brands not mentioned.

the bottle. This reduces the risk of entrance of contaminating particles, and also permits the assembly to rest on the table top while the cap is kept a safe distance above the table top. The top edge of the glass sleeve is turned in to decrease deposition of dust and foreign particles on the filter paper.

With this bottle closure, it is possible to inoculate one or a group of bottles very simply. The closure is unscrewed beforehand, but left on the top of the bottle. After addition of the inoculum, the

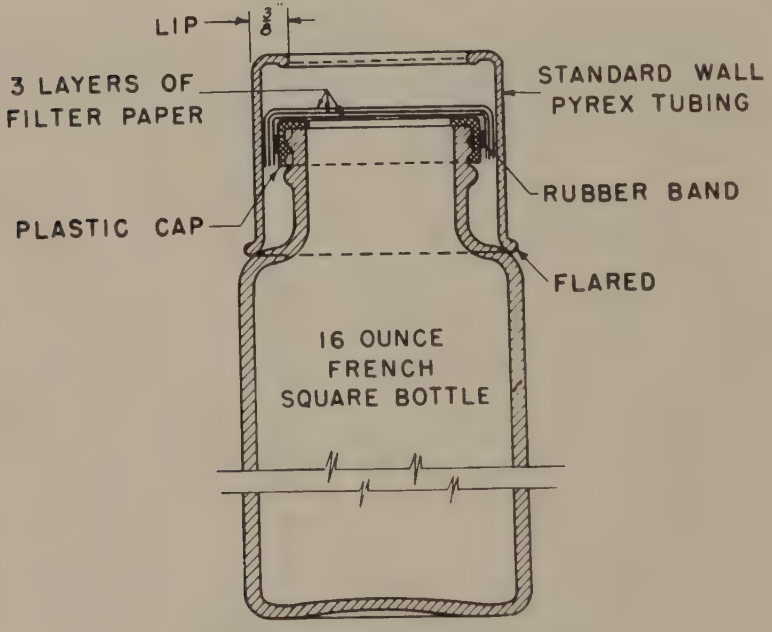

FIG. 1. Bottle with bottle cap assembly.

closure can again be placed on the bottle top, and screwed down later.

Use of this bottle closure has made it possible to obtain excellent spore crops from strains of *Aspergillus niger* and *Aspergillus oryzae* in a short time. Culture of these organisms on media supporting excellent growth in containers not having wide mouths closed with material of good air permeability showed suppressed spore formation.

Media cannot be stored for any length of time in wide-mouth bottles capped either with cotton plugs or the closure described herein without loss of water. We therefore explored the possibility of using the assembly with only a hole drilled in the top of the cap instead of having the entire top removed. This would restrict the loss of water and perhaps have little effect on the spore production of the micro-organism. A clearance between the top of the cap and the filter paper of at least $\frac{1}{16}$ of an inch was effected by a rubber washer $\frac{1}{8}$ inch wide and equal in diameter to the diameter of the cap. Sixteen-ounce French square bottles containing 50 mls. of whole potato media (7% solids) were inoculated with spores of *Aspergilus oryzae* NRRL *458* and incubated for 4 days at 30°C. Spore counts per bottle are shown below.

	Spores per bottle, billions
Closure having 1.5-inch hole (top of cap removed)	9.00
Closure having $\frac{1}{16}$-inch hole in cap	0.85
Closure having $\frac{1}{8}$-inch hole in cap	1.40
Closure having $\frac{1}{4}$-inch hole in cap	2.30
Closure having $\frac{1}{2}$-inch hole in cap	3.00

The spores in the bottles having holes less than 1.5 inches in diameter in the caps were smaller and lacked characteristic color.

The bottle closure described can be constructed easily and can be used over and over again without loss of its effectiveness. For quantity production, a solid plastic piece could be used in place of the glass sleeve and the plastic cap. The filter paper could then be cut to fit the inside of the cap opening, and held against it by a plastic ring or other suitable means. This arrangement would make it convenient to replace the filter paper. A closure of this type should be useful for many containers employed in culture work.—ELMER A. WEAVER, THEONE C. CORDON AND HARRY J. JOHN, Eastern Regional Research Laboratory, Philadelphia 18, Pennsylvania.

A TECHNIQUE OF MOUNTING FUNGAL COLONIES FOR MUSEUM SPECIMENS

In the identification of many fungi in plate culture it is imperative to observe both sides of the colony. It is necessary to see the "reverse" or underside of the growth on agar to observe pigment pro-

duction and diffusion. This is true of many species, especially in the Fungi Imperfecti, as, for example, the Penicillia and Aspergilli. To have the spore-bearing apparatus in its normal arrangement with a three-dimensional configuration is desirable in most cases. Hence, to be able to study both sides of the mounted dried specimen under high-dry magnification with various adjustments of reflected or transmitted light calls for a suitable method of preservation and mounting.

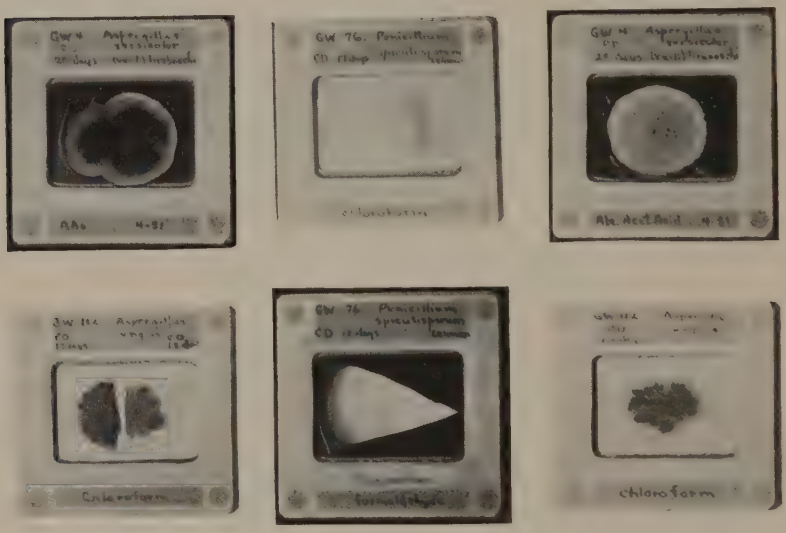

FIG. 1. Specimens showing several types of mountings for different fungi.

The method herein described makes use of 35 mm. mounting frames with one glass used in the frame. The Kodaslide Ready-mounts of the Eastman Kodak Company, the SVE Slide Binders of the Society for Visual Education, Inc., or the Automounts of E. Leitz, Inc. (all termed mounting frame here) serve the purpose. Whole colonies (6–8 days old, depending on the rate of growth) may be mounted in their entirety, or parts of colonies, preferably sectors, can be used where the young colony may not show certain details which are evident in an older one too broad to mount *in toto*. The specimens illustrated in FIG. 1 show several types of mountings of different fungi.

PROCEDURES

1. Growing the colony for mounting

Although any solid agar medium in a Petri dish can be used for growing the organism, one which differentiates certain characters may be more advantageous, such as Czapek-Dox modified agar for the Penicillia. Malt extract, potato dextrose, and corn meal agars are also satisfactory for growth but not always diagnostic. The last-named does not adhere well to the glass surface if it is very granular or has large pieces of the corn meal present.

2. Killing the organism

The use of non-viable mounts eliminates the possibility of contaminating the air with viable spores when studying the materials. Several milliliters of chloroform in the lid of the inverted dish overnight are sufficient to kill the mycelium and the spores. Formaldehyde also can be used as the killing agent, but the resulting product is not as satisfactory because of wetting and matting of the aerial mycelium and crystal formation on the surface. No growth could be obtained from transfers made from specimens treated with chloroform or formaldehyde, whereas material treated with ether or benzene remained viable. Also, some killing agents dissolve pigments from the cells followed by diffusion of color into the agar.

3. Preparation of colony for mounting

When the growing colony has reached a convenient size, a sector or the entire colony can be cut (after killing) from the rest of the agar plate with a large cork borer, a spatula, or an inverted shell vial. The fungus and the adhering agar are placed directly onto the glass slide and air dried. A convenient method to prevent dust accumulation is to place the slide and the mount in an empty Petri dish with the lid ajar. No adhesive, such as albumen fixative, is needed, since the agar acts as such on drying. The mount is dry in 24–48 hours, depending on the age and thickness of the agar, and the water content of the whole mount. When the colony is dried, the glass can be sealed between the two sides of the mounting frame and labeled.

4. Storage of the mounts

A thin cover slip or cover glass may be added over the colony by elevating it with two thin pieces of glass or thick mounting material as balsam at the four corners; however, to be able to examine the specimen under high-dry magnification it seems best not to put a cover above the fungus, which necessitates keeping the museum specimens separated in a "Unifile" slide case where they cannot rub against each other. The uncovered mounts can then be stored individually in any file for 35 mm. slides. Cellulose acetate envelopes are available which can be used to store a greater number of mounts. These may, however, flatten the aerial surface if the mount is excessively thick.

Some fungi with much cottony, floccose hyphae wet and pack tightly, so that these mounts are not as satisfactory as others. To date, mounts have been stored in darkness at room temperature (22–26° C.) for a period of 16 months with no evidence of breaking or fading. Spore colors, including blues and greens, which often fade in dried, unkilled mounts, have in these preparations retained their natural colors. MARLIN A. ESPENSHADE, Department of Botany, George Washington University, Washington, D. C.

DISPOSITION OF NOMINA GENERICA CONSERVANDA PROPOSITA FOR FUNGI

The Special Committee for Fungi adopted at the VII International Botanical Congress at Stockholm five resolutions concerning nomina generica conservanda proposita:

I. That every proposal for conservation not "accompanied by a detailed statement of the cases for and against . . . conservation" [Art. 21, Note 1, of the 3rd edition of the Rules; Art. 24, Note 1, of the new Code] nor having such a statement supplied by a later student, is to be rejected, to be reinstated as a subject for consideration by the Committee only after such a statement is supplied.

The list of proposals so rejected need not be set forth here. It may be that proposals with such statements have escaped the attention of the Committee; if so, these are subject to later action. It is here implied, however, that all proposals submitted before July 1,

1950, and not referred to in succeeding paragraphs are automatically rejected under this first resolution.

II. That every proposal for conservation of a name that would be retained without conservation against the nomina rejicienda proposita is to be rejected.

The following proposals are automatically rejected under this second resolution:

Agaricus vs. Psalliota & Pratella
Auricularia vs. Auricularia & Laschia
Calocera vs. Corynoides
Clitopilus vs. Pleuropus
Collybia vs. Gymnopus
Cordyceps vs. Cordiceps
Cystopus vs. Cystopus
Cyttaria vs. Cyttarium
Flammula vs. Flammula, Gymnopilus, Gymnocybe, Flammopsis, Ryssospora, & Visculus
Gomphus vs. Gomphora
Guepiniopsis vs. Guepinia
Guignardia vs. Carlia
Gymnosporangium vs. Puccinina, Aecidium, & Roestelia
Hericium vs. Hericium
Hexagona vs. Scenidium
Hydnum vs. Dentinum
Hydrocybe vs. Leucopus
Hymenochaete vs. Stereum
Hymenogramme vs. Aschersonia, Junghuhnia, & Laschia
Hypomyces vs. Hypolyssus
Marssonina vs. Marsonia & Marssonia
Melampsora vs. Uredo
Nidularia vs. Granularia
Octaviania vs. Octavianina
Olpidiopsis vs. Pleocystidium & Diplophysa
Omphalina vs. Perona
Panus vs. Rhipidium
Paxillus vs. Rhymovis & Ruthea
Peziza vs. Aleuria
Phallus vs. Ithyphallus
Phellinus vs. Poria
Pholiota vs. Derminus
Phoma vs. Sphaeropsis
Pisomyxa vs. Bryocladium
Plowrightia vs. Dothidella
Podoscypha vs. Craterella
Poria vs. Poria & Physisporus

Porodisculus vs. Enslinia
Pseudolpidium vs. Olpidiopsis
Pterula vs. Pterula
Puccinia vs. Puccinia & Puccinia
Secotium vs. Endoptychum
Sphaeropsis vs. Macroplodia
Stereum vs. Auricularia & Stereum
Telamonia vs. Raphanazon
Tomentella vs. Hypochnus
Tremella vs. Tremella & Gyraria
Trichosporium vs. Alytosporium
Venturia vs. Endostigme
Volvaria vs. Pseudofarinaceus
Xerocomus vs. Versipellis

III. That every proposal for conservation of a name that is a later homonym of a name in current use for a genus in another group is to be rejected; and that consultation is to be immediately undertaken with the appropriate Special Committees concerning all other names that are later homonyms of names in other groups.

The following proposals are automatically rejected under this third resolution:

Galera vs. Galera
Psathyra vs. Psathyra
Sphaerella vs. Sphaerella & Mycosphaerella

IV. That the following names proposed for conservation and about which there seems to be little difference of opinion are to be conserved, such action to be automatically rescinded if their present status is altered by action of the Stockholm Congress on the Rules:

[Note that this list is published in Int. Code Bot. Nomencl. pp. 76–78. 1952. The following corrections in that published list should be noted:

p. 76, right column, last line: for "1823" read "1822."
p. 77, right column, l. 19: after "1843" add "T.: idem."
p. 78, right column, l. 2: for "1: 6" read "1: 7."
p. 77, right column, l. 47, 48: for "Schw. ex Fr.," etc., read "Schw., Naturf. Ges. Leipzig Schr. 1: 108. 1822."
p. 78, left column, l. 2, 3: for "Pers. ex Fr.," etc., read "Pers., Mycol. Eur. 1: 141. 1821."

p. 78, left column, l. 4, 5 and l. 6, 7: for "Tode ex Fr.," etc., read "Pers. ex Hook., Fl. Scot. **2**: 9. 1821."

Of these, the first three are typographical errors, and the last three are results of the application of the new Art. 22 f. None have any nomenclatorial consequences.]

Nomina conservanda	Nomina rejicienda
Aleurodiscus Rab. ex Cooke, Grevillea **3**: 136. 1875.—T.: *Thelephora amorpha* (Pers.) Fr., El. Fung. **1**: 183. 1828.	*Cyphella* Fr., Syst. Mycol. **2**: 201. 1822.—T.: *C. digitalis* [A. & S.] ex Fr., l.c.
Calvatia Fr., Summ. Veg. Scand. 442. 1849.—T.: *Bovista craniiformis* Schw., Amer. Phil. Soc. Trans. II **4**: 256. 1832.	*Langermannia* Rostk. in Sturm, Deutschl. Fl. III **5** (18): 23. 1839.—T.: *Lycoperdon giganteum* Batsch ex Pers., Syn. Meth. Fung. 140. 1801. *Hippoperdon* Mont., Ann. Sci. Nat. Bot. II **17**: 121. 1842.—T.: *H. crucibulum* Mont., l.c.
Clavaria Vaill. ex Fr., Syst. Mycol. **1**: 465. 1821.—T.: *C. fragilis* Pers. ex Fr., op. cit. 484.	*Clavaria* Stackh., Ner. Brit. 2 ed. *x*. 1816.—T.: *Fucus clavatus* Lamour., Dissert. Esp. Fucus 22. 1805.
Daldinia Ces. & Not., Soc. Crittogam. Ital. Comm. **1**: 197. 1863.—T.: *Sphaeria concentrica* Pers. ex Hook., Fl. Scot. **2**: 4. 1821.	*Peripherostoma* Gray, Nat. Arr. **1**: 513. 1821.—T.: idem. *Stromatosphaeria* Grev., Fl. Edin. *lxxiii*. 1824.—T.: idem. *Hemisphaeria* Nees ex Klotzsch, Acad. Caes. Leop. Carol. Nat. Cur. Nova Acta **19**, suppl. 1: 241. 1843.—T.: idem.
Marasmius Fr., Fl. Scan. 339. 1835.—T.: *Agaricus rotula* Scop. ex Fr., Syst. Mycol. **1**: 136. 1821.	*Micromphale* Gray, Nat. Arr. **1**: 621. 1821.—T.: *M. venosum* [Pers.] ex Gray, op. cit. 622.
Melanogaster Corda in Sturm, Deutschl. Fl. III **3** (11): 1. 1831.—T.: *M. tuberiformis* Corda, l.c.	*Bullardia* Jungh., Linnaea **5**: 408. 1830.—T.: *B. inquinans* Jungh., l.c.

Nomina conservanda	Nomina rejicienda
Panus Fr., Epicr. 396. 1838.—T.: *Agaricus conchatus* Bull. ex Fr., Syst. Mycol. **1**: 181. 1821.	*Pleuropus* [Pers.] ex Gray, Nat. Arr. **1**: 615. 1821.—T.: *Pl. fornicatus* [Pers.] ex Gray, l.c.
Pleurotus (Fr.) Kummer, Führer Pilzk. 24. 1871.—T.: *Agaricus ostreatus* Jacq. ex Fr., Syst. Mycol. **1**: 182. 1821.	*Crepidopus* [Nees] ex Gray, Nat. Arr. **1**: 616. 1821.—T.: idem. *Resupinatus* [Nees] ex Gray, op. cit. 617.—T.: *Agaricus applicatus* Batsch ex Fr., op. cit. 192. *Pterophyllus* Lév., Ann. Sci. Nat. Bot. III **2**: 178. 1844.—T.: *Pt. Bovei* Lév., l.c. *Hohenbuehelia* Schulz., Zool.—Bot. Ges. Wien Verh. **16**: Abhandl. 45. 1866.—T.: *A. petaloides* Bull. ex Fr., op. cit. 183.
Septobasidium Pat., Jour. de Bot. **6**: 63. 1892.—T.: *S. velutinum* Pat., op. cit. 62.	*Gausapia* Fr., Syst. Orb. Veg. 302. 1825.—T.: *Thelephora pedicellata* Schw., Naturf. Ges. Leipzig Schr. **1**: 108. 1822. *Glenospora* Berk. & Desm., Hort. Soc. Lond. Jour. **4**: 255. 1849.—T.: *G. Curtisii* Berk. & Desm., l.c. *Campylobasidium* Lagerh. ex Ludw., Lehrb. nied. Kryptogam. 474. 1892.—T.: deest.
Stagonospora (Sacc.) Sacc., Syll. Fung. **3**: 445. 1884.—T.: *Hendersonia paludosa* Sacc. & Speg., Michelia **1**: 353. 1878.	*Hendersonia* Berk., Ann. Mag. Nat. Hist. I **6**: 430. 1841.—T.: *H. elegans* Berk., l.c.
Tomentella Pers. ex Pat., Hym. Eur. 154. 1887.—T.: *Thelephora ferruginea* Pers., Mycol. Eur. **1**: 141. 1822.	*Caldesiella* Sacc., Michelia **1**: 6. 1887.—T.: *C. italica* Sacc., op. cit. 7.
Tuberculariа Pers. ex Hook., Fl. Scot. **2**: 9. 1821.—T.: *T. vulgaris* Pers. ex Hook., Fl. Scot. **2**: 9. 1821.	*Tuberculariа* Wigg., Primit. Fl. Holsat. 87. 1780.—T.: *Lichen ericetorum* Scop., Fl. Carniol. 2 ed. **2**: 359. 1772.

Nomina conservanda	Nomina rejicienda
Uromyces (Link) Unger, Exanth. Pfl. 277. 1833.—T.: *Uredo appendiculata* Pers., Syn. Meth. Fung. 221, 222. 1801.	*Nigredo* Rouss., Fl. Calv. 47. 1806.—T.: *Uredo betae* Pers., op. cit. 220. *Caeomurus* [Link] ex Gray, Nat. Arr. **1**: 541. 1821.—T.: *U. appendiculata,* l.c. *Pucciniola* March., Bijdr. Nat. Wet. **4**: 47. 1829.—T.: *P. diadelphiae* March., op. cit. p. 48.

V. That as soon as the Rules as amended by the Stockholm Congress are available, all proposals for conservation not adopted or rejected under the foregoing paragraphs [I–IV] are to be referred to the members of the Committee for a vote, and that six months after the date of mailing of blank ballots the votes returned are to be recorded and the decisions of the Committee published.

By a written vote, completed in February, 1952, the Committee adopted the following resolution:

The assent of three-fifths of the members voting shall be necessary for the adoption of any proposal for conservation.

By the same written ballot the following proposals for conservation were adopted:

Nomina conservanda	Nomina rejicienda
Amanitopsis Roze, Soc. Bot. France Bul. **23**: 50. 1876.—T.: *Agaricus vaginatus* Bull. ex Fr., Syst. Mycol. **1**: 14. 1821.	*Vaginata* Nees ex Gray, Nat. Arr. **1**: 601. 1821.—T.: *V. livida* Gray, l.c.
Amphisphaeria Ces. & Not., Soc. Crittogam. Ital. Com. **1**: 223. 1863.—T.: *Sphaeropsis conica* Lév. in Demidoff, Voy. Russ. **2**: 112. 1842.	*Sphaeropsis* Lév., l.c.—T.: idem.
Aschersonia Mont., Ann. Sci. Nat. Bot. iii **10**: 121. 1848.—T.: *A. taitense* Mont., l.c. p. 122.	*Aschersonia* Endl., Gen. Pl. Suppl. **2**: 103. 1842.—T.: *Laschia crustacea* Jungh., Batav. Gen. Kunst. Wetensch. Verh. 17 Deel, III Stuck: 75. 1838.
Candida Berkh., De schimmelgesl. Monilia 41. 1923.—T.: *C. vulgaris* Berkh., l.c. p. 42.	*Syringospora* Quinq., Arch. Physiol. Norm. Path. **1**: 293. 1868.—T.: *Oidium albicans*

Nomina conservanda	Nomina rejicienda
	Rob., Hist. Nat. Veg. Paras. 502. 1853. *Parendomyces* Queyr. & Lar., Soc. Méd. Hôp. Paris Bull. Mem. iii **28**: 136. 1909.—T.: *P. albus* Queyr. & Lar., l.c. *Parasaccharomyces* Beurm. & Gong., Trib. Méd. **42**: 502. 1909 [? Nomen provisorium. Cf. op. cit. p. 518].—T.: deest; sed conf. pp. 521, 524, *"P. Sambergeri"* (nom. nud.). *Pseudomonilia* Geiger, Centralbl. Bakt. 134. 1910.—T.: *P. albomarginata* Geig., l.c. p. 135.
Coniothyrium Corda, Icon. Fung. **4**: 38. 1840.—T.: *C. Palmarum* Corda, l.c.	*Clisosporium* Fr., Syst. Mycol. **3**: 334. 1832.—T.: *Cl. lignorum* Fr., l.c. p. 335.
Conocybe Fayod, Ann. Sci. Nat. Bot. vii **9**: 357. 1889.—T.: *Agaricus tener* Schaeff. ex Fr., Syst. Mycol. **1**: 265. 1821.	*Raddetes* Karst., Hedwigia **26**: 112. 1887.—T.: *R. turkestanicus* Karst l.c. *Pholiotina* Fayod, l.c. p. 359.—T.: *A. blattarius* Fr., l.c. p. 246. *Pholiotella* Speg., Acad. Sci. Cordoba Bol. **11**: 412. 1889.—T.: *Pholiotella blattariopsis* Speg., l.c.
Gautieria Vitt., Monogr. Tuber. 25. 1831.—T.: *G. morchellaeformis* Vitt., l.c.	*Gautiera* Raf., Med. Fl. **1**: 202. 1828.—T.: *Gaultheria procumbens* L., Sp. Pl. **1**: 395. 1753.
Gyromitra Fr., Summ. Veg. Scand. 346. 1849.—T.: *Helvella esculenta* Pers., Mycol. Eur. **1**: 212. 1822.	*Gyrocephalus* Pers., Soc. Linn. Par. Mém. **3**: 77. 1824.—T.: *H. sinuosa* Brond., Soc. Linn. Paris Mem. **3**: [74]. 1824.
Mutinus Fr. Summ. Veg. Scand. 434. 1849.—T.: *Phallus caninus* Pers. Syn. Meth. Fung. 245. 1801.	*Aedycia* Raf., Jour. Bot. Soc. Bot. [Desvaux] **1**: 222. 1808.—T.: *A. rubra* Raf., l.c. *Ithyphallus* Gray, Nat. Arr. **1**:

Nomina conservanda	Nomina rejicienda
	675. 1821.—T.: *P. caninus*. *Cynophallus* (Fr.) Corda, Icon. Fung. **5**: 29. 1842.—T.: *P. caninus*.
Mycobonia Pat., Soc. Mycol. Fr. Bul. **10**: 76. 1894.—T.: *Peziza flava* Swartz ex Fr., Syst. Mycol. **2**: 161. 1822.	*Hirneola* Fr., Syst. Orb. Veg. 93. 1825.—T.: idem.
Panaeolus (Fr.) Quél., Soc. Émul. Montbeliard Mém. ii **5**: 151. 1872.—T.: *Agaricus papilionaceus* Bull. ex Fr., Syst. Mycol. **1**: 301. 1821, emend. Fr., Epicr. 236. 1838.	*Coprinarius* (Fr.) Kummer, Führer Pilzk. 20. 1871.—T.: idem.
Phoma Sacc., Michelia **2**: 4. 1880.—T.: *P. herbarum* Westend. ex Sacc., Syll. Fung. **3**: 133. 1884.	*Phoma* Fr. ex Fr., Syst. Mycol. **2**: 546. 1823.—T.: *P. pustula* (Pers.) ex Fr., l.c. p. 547.
Pseudographis Nyl., Soc. Sci. Nat. Cherbourg Mém. **3**: 190. 1855.—T.: *Hysterium elatinum* (Ach.) Pers. ex Fr., Syst. Mycol. **2**: 584. 1823.	*Krempelhuberia* Massal., Geneac. Lichen. 34. 1854.—T.: *K. Cadubriae* Massal., l.c. p. 15.
Rhabdospora (Dur. & Mont. ex Sacc.) Sacc., Syll. Fung. **3**: 578. 1884.—T.: *Septoria Oleandri* Dur. & Mont., Expl. Sci. Alger. **1**: 593. 1849.	*Filaspora* Preuss, Linnaea **26**: 718. [1855].—T.: *F. perithecioides* Preuss, l.c.
Rhipidium Cornu, Soc. Bot. Fr. Bul. **18**: 58. 1871.—T.: *R. interruptum* Cornu, l.c.	*Rhipidium* Wallr., Fl. Crypt. Germ. **2**: 742. 1833.—T.: *Agaricus stipticus* Bull. ex Fr., Syst. Mycol. 1: 188. 1821.
Urocystis Rabenh. ex Fuckel, Nass. Ver. Naturk. Jahrb. **23–24**: 41. [1870].—T.: *Erysibe occulta* Wallr., Fl. Crypt. Germ. **2**: 212. 1833.	*Polycystis* Lév., Ann. Sci. Nat. Bot. iii **5**: 269. 1846.—T.: *Caeoma pompholygodes* Schlecht., Linnaea **1**: 248. 1826. *Tuburcinia* Fr., Syst. Mycol. **3**: 439. 1832.—T.: *Rhizoctonia*. *Orobanches* Mérat, Nouv. Fl. Paris 2 ed. **1**: 135. 1821.

By the same written ballot the following proposals were rejected:

Acrotheca vs. Gomphinaria
Acrothecium vs. Cordana & Cacumisporium
Aposphaeria vs. Aposphaeria & Coniothyrium
Barya vs. Barya
Calodon vs. Hydnellum
Capnodium vs. Fumago, Apiosporium, & Polychaeton
Cladoderris vs. Cymatoderma
Clitopilus vs. Pleuropus
Coniosporium vs. Coniosporium
Coniothyrium vs. Clisosporium & Coniothyrium
Corticium vs. Phlebia, Corticium, Ricnophora, Athelia & Hyphoderma
Cortinarius vs. Cortinaria
Craterellus vs. Craterella
Dichaena vs. Heterographa
Dictyolus vs. Leptoglossum & Leptotus
Empusa vs. Empusa
Guepinia vs. Gyrocephalus & Phlogiotis
Guepinia vs. Guepinia
Hendersonia vs. Sporocadus & Hendersonia
Hirneola vs. Hirneola & Laschia
Hymenochaete vs. Hymenochaete
Hymenula vs. Hymenella
Hypochnus vs. Lyomyces
Hypolyssus vs. Caripia
Hypospila vs. Phoma
Lachnocladium vs. Eriocladus
Macowanites vs. Macowania & Hypochanum
Massaria vs. Splanchnonema
Mastomyces vs. Topospora
Monilia vs. Monilia
Monotospora vs. Monotospora
Montagnites vs. Montagnea
Mutinus vs. Aedycia
Mycoleptodon vs. Odontia
Mytilidion vs. Mytilinidion
Nummularia vs. Biscogniauxia, Nummularia, & Numularia
Ophiostoma vs. Endoconidiophora
Pactilia vs. Achitonium & Leucosporium
Peniophora vs. Corticium & Hyphoderma
Phleospora vs. Septoria
Phlyctidium vs. Phlyctidium
Pilacre vs. Phleogena
Polystictus vs. Coltricia & Strilia
Ramaria vs. Ramaria & Cladaria
Ramularia vs. Ramularia & Cylindrospora
Rhabdospora vs. Filaspora

Panellus vs. Rhipidium
Rhodophyllus vs. Acurtis, Entoloma, Leptonia, Nolanea, Eccilia, & Claudopus
Septoria vs. Septoria
Sordaria vs. Schizothecium, Enterobotyrum, Podospora, Malinvernia, & Pleurage
Spegazzinula vs. Dubitatio
Sphaeropsis vs. Macroplodia & Sphaeropsis
Stigmatea vs. Ascospora
Stigmea vs. Ascospora
Teichospora vs. Strickeria & Sphaeria
Thyrsidium vs. Cheirospora, Hyperomyxa, & Myriocephalum
Trematostoma vs. Clypeothecium
Tremellodon vs. Pseudohydnum & Hydnogloea
Trichosporium vs. Colletosporium & Alytosporium
Tromera vs. Biatorella & Sarea
Volvaria vs. Volvaria

The vote on the several proposals to conserve *Tricholoma* was inconclusive, and another vote is being taken.

It should be noted that the lists of rejected proposals are made up of abbreviated entries. In different proposals different types may have been considered for a single genus, and the nn. rejic. prop. may have been distributed among several separate proposals.

It should also be noted that the proposals approved by the written vote of the Committee are not thereby finally adopted. They are now submitted to the Advisory Board and the General Committee of Botanical Nomenclature (cf. Taxon **1**: 95) whose action will be reported in Taxon; if favorable action is received, they are tentatively adopted, and the conserved names may be retained "pending the decision of the next International Botanical Congress" (Int. Rules, 3 ed., Art. 22; Code, Art. 25). As a matter of practice, there seems no reason to anticipate the subsequent rejection of any of these proposals; all those known to conflict with names used in groups other than the Fungi have already been submitted (under the Committe's Resolution III) to the secretaries of the appropriate special committees.

Copies of new proposals for conservation of generic names of Fungi (with correct citation of names of genera and of types, and with arguments for and against conservation) should be sent to Prof. J. Lanjouw, Secretary, General Committee of Botanical Nomenclature, Lange Nieuwstraat 106, Utrecht, Netherlands, and

to the undersigned. To facilitate study and discussion before the 1954 Congress by those interested, authors are urged to publish such new proposals before September, 1953.—DONALD P. ROGERS, Secretary, Special Committee for Fungi, The New York Botanical Garden, Bronx Park, New York 58, N. Y., U. S. A.

SYDOWIA

Sydowia, the Journal of Taxonomic Mycology, established by Dr. Franz Petrak of Vienna, Austria, following the death of H. Sydow, to succeed the Annales Mycologici is now in its 7th volume. A large amount of valuable taxonomic material has been issued in these volumes, much of it by Dr. Petrak himself. As might be expected in these trying times of rapidly increasing costs, the journal is experiencing financial difficulties and it is suggested that additional subscriptions will afford effective help. Institutional subscriptions are particularly desired. The journal is published by Ferdinand Berger, Horn, n-ö, Austria, at 40 Swiss francs per volume.

REVIEWS

Précis de Mycologie. Mycologie générale. Mycologie humaine et animale. Techniques, by M. Langeron. Deuxième edition, revue et augmentée, by R. Vanbreuseghem. ix–703 pp., 461 figs. Masson & Cie., Paris, 1952. Price Fcs. 4800 unbound (about $13.75); fcs. 5400 bound (about $15.50).

The first edition of Langeron's text, published in 1945, met with wide recognition. Before his death, Dr. Langeron had made notes in preparation for a revised edition. So far as available, these were turned over to Dr. Vanbreuseghem and the preparation of the new edition was entrusted to him. The new work is greatly enlarged, more so than the increased number of pages would suggest, since the larger page of the new edition accommodates approximately half as much material again as did a page of the first edition.

The general form of the older work is retained. The first nine chapters, comprising over half of the volume, constitute Part I, General Mycology. The first chapter, in which Langeron argued for the inclusion of the fungi in the Protista, is retained with little change. This is followed by a review of the classification of the fungi and an extensive treatment of their morphology, spore dispersal and sexuality. Part II, Techniques, is arranged as a single long chapter, in which the valuable information on this subject in the first edition is repeated, with some revision, and expanded. Part III, Medical Mycology, is entirely new and is the work of Dr. Vanbreuseghem. In its eighteen chapters, the principal groups of diseases are treated in alphabetical order, beginning with those due to Actinomycetes and ending with the sporotrichoses. There is no general bibliography, but in the first two parts footnotes are used freely and in the third part each chapter has its special bibliography.

The book is beautifully printed and the illustrations are carefully selected and clearly reproduced. It deserves a place in every laboratory where fungi are seriously studied.—G. W. M.

MICOLOGIA. MORFOLOGIA, BIOLOGIA, EXPERIMENTACION, by Luis C. Verna and Federico J. Herrero. 740 pp., 286 figs. Editorial "El Ateneo," Buenos Aires. 1952. Price 120 pesos (about $8.50).

The attempt is made to cover, in a volume of convenient size, the entire field of mycology. The first six chapters deal concisely with the history of mycology, the systematic position of the fungi and fungal morphology, cytology, biology, chemical composition, sexuality and genetics. A long chapter on mycological techniques follows. About a third of the book, divided into five chapters, is devoted to classification. Two additional chapters provide rather full discussion of human pathogens and of the industrial applications of fungi. A glossary, bibliography and index complete the volume. Without neglect of theoretical foundations, the emphasis is practical. This is reflected in the full treatment given to certain important groups, notably to the yeasts, the Aspergilli and the Penicillia.

The book is marred by too many misprints and by the misspelling of the names of authors ("Taxter," "Buillard," "Dangear," "Pearson" [for Persoon], "Engler y Plant") and of scientific names ("Zoofage," "Olipdiopsis," "Tricophyton," "Licoperdum," to cite only a few). Few of these will cause confusion, but it is to be hoped that they may be corrected in future printings. Some of the figures, especially those reproducing photographs, could be vastly improved. Figure 64, p. 146, is misleading in showing only two nuclei as the result of meiosis, even though in some cases only two of the resulting four nuclei actually function.

This is the most comprehensive modern treatment of the fungi in Spanish which has come to my attention. The authors, both of whom are professors in the National University of Tucumán, are to be congratulated on their achievement. The book will be useful to all working mycologists but it is particularly recommended to graduate students in English-speaking countries, who will find in it not only much valuable information but an extremely helpful introduction to mycological discussion in a language in which the mycological literature is becoming increasingly important.—G. W. M.

MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

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SCREENING TESTS DESIGNED TO DISCOVER ANTIBIOTICS SUITABLE FOR PLANT DISEASE CONTROL [1]

G. J. STESSEL, CURT LEBEN AND G. W. KEITT [2]

(WITH 2 FIGURES)

Most of the antibiotics now used in medicine have resulted from systematic searches. These materials have passed successfully a number of screening tests, many of which were devised to select antibiotics that would control bacterial or virus diseases of man and animals. Many of these materials have been tested for plant disease control and merit further work. However, in view of the remarkable accomplishments with antibiotics in medicine and of the evidence that antibiotics may be obtained in great variety and high potency, it seemed desirable to seek kinds especially adapted to plant disease research by employing screening techniques oriented toward plant pathology, rather than toward medicine. The present paper is an account of such a screening program. Especial emphasis was placed on seeking antifungal antibiotics, since most of the plant diseases for which chemicals are used at present are incited by fungi.

[1] Supported in part by the Research Committee of the Graduate School with funds supplied by the Wisconsin Alumni Research Foundation. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Accepted for publication Nov. 2, 1952.

[2] We wish to express our appreciation to Eugene Herrling, who took the photographs.

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The work consisted of four major screening steps designed to find stable antibiotics that could be produced in quantity and that would be effective against a range of plant pathogens. The steps were (a) selection of antagonistic microorganisms from the soil, using plant pathogenic fungi as test organisms, (b) screening the selected antagonists on the basis of inhibition of a number of plant pathogens, (c) production of antibiotics by the antagonists in liquid media, and (d) studies on the stability of the antibiotics produced by selected organisms. A short account of most of this work has already appeared (**9**).

SELECTION OF ANTAGONISTIC MICROORGANISMS FROM SOIL

Primary selection tests consisted of spraying agar Petri plates containing colonies of soil microorganisms with standardized conidial suspensions of three or four fungi shown in preliminary tests to be mutually nonantagonistic. After incubating the plates, colonies that were surrounded by clear zones of inhibition of the test organisms were singled out for further study. An account of this procedure is given below.

During the autumn of 1948, 40 soil samples were collected in and near Madison, Wisconsin, and stored at 8° C until used. These samples were added to yeast extract-glucose agar [3] in dilutions previously found to give approximately 30 to 50 colonies per plate; final dilutions of soils in agar were 1 : 200,000 to 1 : 20,000,000. Approximately 20 ml of medium was used in each plate. Forty plates from each soil were incubated at 26° for 7–14 days.

After the incubation period plates were inoculated with a mixed inoculum using a spraying technique similar to those of Stansly (8) and Wilska (12). In the early part of the work each plate was sprayed for about three seconds with a standardized spore suspension containing nearly equal concentrations of conidia of *Glomerella cingulata* (Stonem.) Spauld. & v. Schrenk, *Colletotrichum circinans* (Berk.) Vogl., and *Helminthosporium victoriae* M. & M. For the major part of the work, conidia of *Verticillium albo-atrum* Reinke & Berth. were also present in the inoculum.

[3] Per l.; Bacto yeast extract, 2 gm; glucose, 10 gm; agar, 17 gm; pH after autoclaving, 6.0 to 6.4.

Conidia for spray suspensions were obtained from cultures of the fungi that had been grown on potato-glucose agar [4] at 24° for 7–14 days. The sprayed plates were incubated at 26° for two days. Soil colonies that were antagonistic to the plant pathogens were easily located at that time because of the surrounding zone of inhibition (FIG. 1). Stock cultures were made from colonies that produced large clear zones free from growth of any of the four pathogens.

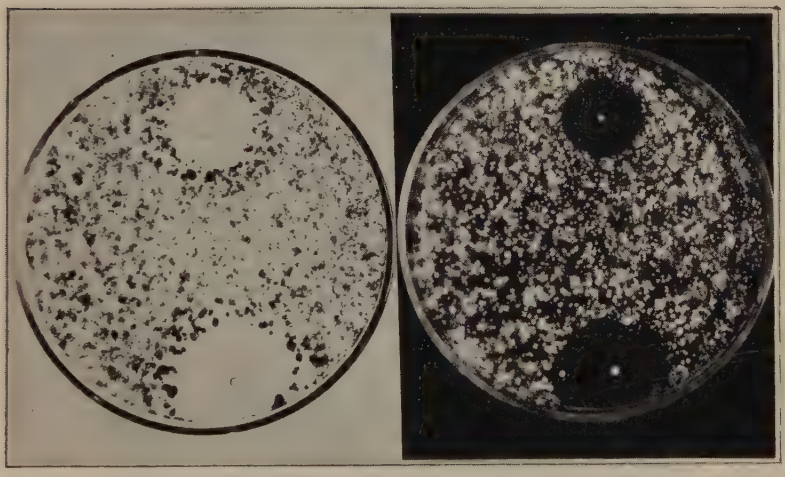

FIG. 1. A plate culture showing the inhibition of four mutually non-antagonistic plant pathogenic fungi by two organisms from soil. The picture with the light background shows growth of the two dark pathogens, *C. circinans* and *H. victoriae,* and the one with the dark background shows growth of the two light organisms, *V. albo-atrum* and *G. cingulata.*

From approximately 70,000 soil colonies that were tested by the procedure, 170 antagonistic organisms were isolated. Approximately 80 per cent of these were actinomycetes. One hundred and two of these antagonistic organisms remained for further work after the elimination of similar, mixed, or contaminated cultures.

[4] Per l.: Extract from 200 gm of sliced, peeled potatoes steamed one-half hour in 500 ml distilled water; glucose, 25 gm; agar, 17 gm; pH 6.7.

SCREENING OF ANTAGONISTS ON THE BASIS OF INHIBITION OF PLANT PATHOGENS

The second step in the screening process, the "agar streak-spray test," was a study of the relative inhibition of a number of plant pathogens by the selected antagonistic microorganisms. The test was made by streaking agar plates with an antagonistic organism, incubating the plates, spraying individual plates with one of the

FIG. 2. The antagonism of *C. circinans* by *Streptomyces* sp. from soil.

plant pathogens, and after a further period of incubation, noting the width of the clear zone between the growth of the antagonistic organism and that of the plant pathogen (FIG. 2). The details of the test follow.

Water suspensions of antagonistic isolates were made from yeast extract-glucose agar [3] cultures grown at 26° for 7–14 days. One loopful of a given suspension was streaked on one side of a yeast extract-glucose agar plate (20 ml of medium per plate). Twenty-five or 30 plates were streaked with each antagonistic isolate. Plates were incubated at 26° for four days, and 20 plates contain-

ing the most uniform growth of each isolate were selected. Two plates of each antagonistic organism were then sprayed with a standardized suspension of one of the pathogens; in all, 10 pathogens were sprayed on the 20 plates. Sprays were prepared from cultures that had grown on potato-agar [4] at 24° for 7–14 days when fungi were used, and when bacteria were employed as test organisms, cell suspensions were derived from potato-glucose agar cultures that had been incubated 48 hours at 24°. Sprayed plates were incubated at 26° for 60–72 hours, and the distance of complete inhibition of growth of the pathogen from the antagonist was measured.

All 102 of the antagonistic isolates obtained as recorded in the first section of the paper were included in agar streak-spray tests. On the basis of the degree of inhibition of the 10 plant pathogens, 22 of the antagonists were selected for further study. These isolates were tested a second time by means of agar streak-spray tests with the 10 phytopathogens. On the basis of both tests, 21 of the isolates were selected for further studies. The plant pathogens used and examples of the degree of inhibition obtained in the second trial are given in TABLE I.

TABLE I

INHIBITION OF CERTAIN PLANT PATHOGENS BY SELECTED ANTAGONISTS IN AGAR STREAK–SPRAY TESTS

Plant pathogen	Width of clear zones produced by stated antagonist:[a]		
	A67[b]	*A102*	*A158*[c]
	mm	mm	mm
Agrobacterium tumefaciens (Smith & Towns.) Conn.	35	15	0
Colletotrichum circinans (Berk.) Vogl.	65	38	29
C. pisi Pat.	30	25	25
Erwinia amylovora (Burr.) Winslow *et al.*	57	12	20
Fusarium oxysporum f. *lycopersici* (Sacc.) S. & H.	15	19	20
F. oxysporum f. *pisi* (Linford) S. & H.	17	18	20
Glomerella cingulata (Stonem.) Spauld. & v. Schrenk	24	24	25
Helminthosporium victoriae M. & M.	65	28	27
Sclerotinia fructicola (Wint.) Rehm	18	20	30
Verticillium albo-atrum Reinke & Berth.	22	31	30

[a] Each entry is the average from two plates.
[b] Produces toximycin (11).
[c] Produces helixin (6).

THE PRODUCTION OF ANTIBIOTICS BY ANTAGONISTS IN LIQUID MEDIA

The purpose of the third step in the screening procedure was to determine which of the 21 selected antagonistic isolates could produce antibiotics in shaken liquid media, since liquid cultures were necessary for obtaining sufficient amounts of antibiotics for fractionation and further study.

To determine whether an antibiotic was present in the liquid media in which the microorganisms had grown, the Petri plate assay method of Dunshee *et al.* (1), employing the fungus, *G. cingulata,* was used with certain modifications. Blotting paper discs were replaced by "penicylinders." The latter were placed on a 10 ml base layer of agar and sealed in place with 5 ml of agar seeded with the fungus. Each sample was tested by filling six cylinders, three in each of two plates. Plates were incubated at 26° for 24–35 hours and the size of inhibition zones noted.

Five hundred ml Erlenmeyer flasks containing 100 ml of liquid medium per flask were used to culture the antagonistic isolates. These were divided into three groups of seven isolates each, and each group was tested at a different time. Flasks were shaken for four days at 26° on a reciprocating shaker making 65 cycles per min. with a 15 cm stroke. The media used in the experiments are given in TABLE II; three replicate flasks were used in each antagonist-medium combination. Suspensions for seeding the flasks were made from cultures of the antagonists that had grown on yeast extract-glucose agar[3] in bottle slants at 26°. Cultures of actinomycetes were 7–14 days old; cultures of isolate *A67* (a bacterium) were 48 hours old. Inoculation was at the rate of 5 ml of spore or cell suspension per flask. After the fermentation period, samples were prepared for assay by removing the cells and sediment either by filtration, using pipette, containing a cotton-filled base, or by centrifugation.

It was found that 16 of the 21 organisms produced diffusible antibiotic materials, as indicated by the production of inhibition zones, when grown in one or more media. The 12 isolates producing zones of complete inhibition of 17 mm or more in diameter were used in a second fermentation test; the medium employed for

TABLE II

MEDIA USED IN TESTS FOR PRODUCTION OF ANTIBIOTICS BY ANTAGONISTS [a]

Ingredient	Gm per liter of ingredient in stated medium:				
	A	B	C	D	F
Beef extract	—	—	—	—	3
$CaCO_3$	—	1.5	5	1.5	—
Corn steep[b]	—	50	10	—	—
Glucose	10	10	20	20	10
Peptone	—	—	—	—	10
Soybean meal	—	—	20	40	—
Yeast extract	2	—	—	—	—

[a] Usual pH of media after autoclaving: A, 6.2; D, 7.2; and F, 6.2. Media containing corn steep (B, C) adjusted to pH 7 before adding $CaCO_3$ and autoclaving. Final pH of these media usually 7.1–7.2.
[b] Solids, 50–60 per cent.

each organism was that most favorable for antibiotic production in the first test. On the basis of the results of both of these tests, as well as the results of the agar streak-spray tests, 8 of the 12 isolates were selected for further study.

STABILITIES OF ANTIBIOTICS PRODUCED BY ANTAGONISTS

The property of an antibiotic to retain its potency under different conditions may determine the methods by which a crude material is purified and its ultimate usefulness in plant disease studies. Culture centrifugates of the eight antagonists previously selected were subjected to a series of tests designed to give some assessment of the stability of the antibiotics produced by these organisms. In part the procedures suggested by Johnson *et al.* (2) were used.

The stability of the antibiotics in culture centrifugates at different pH levels was studied by adjusting the centrifugates to pH 6 and removing five ml. aliquots, which in turn were adjusted to five pH levels from 2 to 10, inclusive. Samples were held at the variout levels for four hours at 29–30°, readjusted to pH 6, made to 10 ml., and then assayed. A dosage-response curve was constructed for the antibiotic produced by each isolate on the basis of inhibition by four two-fold dilutions of the original centrifugate at pH 6; activity of treated samples was determined by interpolation of this curve.

On the basis of the assay results a curve was plotted for each culture centrifugate, using as coordinates the treatment pH and the percentage of the activity remaining after treatment. From this curve the critical pH range (the range over which at least 50 per cent activity was retained) and the optimum pH (the pH for greatest stability) were determined.

To test heat stability, aliquots of culture centrifugates at the optimum pH value were held at 100° for periods of from three minutes to 20 hours; other aliquots were autoclaved. Activity of treated samples was determined by means of dosage-response curves based on activity of different dilutions of untreated samples.

Time stability studies were made on centrifugates that were adjusted to the optimum pH value and stored at 8°, 26°, or 36° after the addition of a small amount of toluene as a preservative. Samples were assayed at intervals up to the time the sample had lost 50 per cent of its original activity or until the experiment was concluded, at 60 days. Decrease in activity of samples at 26° and 36° was calculated at each sampling time on the basis of a dosage-response curve produced by the sample kept at 8°. At each sampling time the approximate decrease in activity of the 8° sample was estimated by reference to the dosage-response curve that had been prepared for this sample at the start of the experiment. Suitable corrections were made in the calculation of the loss in activity of samples at 26° and 36°.

On the basis of the three types of stability tests it was concluded that six of the eight antagonists produced antibiotics that were of sufficient interest for further study. Two of these materials seemed to have special merit; these were later named helixin and toximycin. Of the two antagonists that were not selected for further study, one, a *Streptomyces* species, was eliminated because it was identical with or similar to the *Streptomyces* sp. that produces antimycin (4), since the morphology of the organisms, their growth habits on a number of media, and the properties of the antibiotics produced by them were remarkably similar. No attempts were made to isolate antimycin A from cultures of the organism obtained in the present work.

The six organisms that passed the screening tests were studied determinatively; it was shown that five belonged to the genus *Streptomyces* Waksman & Henrici, and that the sixth was *Bacillus subtilis* Cohn *emend.* Prazmowski. The morphological, cultural, and physiological characteristics of four of the *Streptomyces* species and of *B. subtilis* (our isolate *A67*) are given in detail elsewhere (**10**). The fifth *Streptomyces* isolate and the antibiotics it produces, the helixins, have been described in other papers (**6, 7**).

DISCUSSION

Isolation and screening procedures were designed and employed to search for antibiotics that could be used in plant disease control work. It seemed reasonable to expect that materials selected by these methods would be sufficiently stable for ordinary fractionation procedures and for tests with plant diseases. Because four plant pathogenic fungi were used in the primary isolation work and fungi were used in the second selection step, it was hoped also that the antibiotics that passed all of the screening tests would have activity against a number of fungi. Other work (**3, 5, 6, 7, 11**) has shown that two of the selected antibiotics that have been given further study are active against many fungi, possess reasonable stability, and have potentialities for plant disease control work. It would seem, therefore, that systematic studies of the type described in this paper should be of value in discovering antibiotics that may be of interest to the plant pathologist. These materials may also merit investigation in other fields, since they may act on groups of organisms (*e.g.*, the fungi) rather than individual species or lines.

SUMMARY

Screening methods aimed at finding organisms that produce stable antibiotics which inhibit a range of phytopathogens are described. Agar plates, each containing colonies of 30–50 soil microorganisms, were sprayed with four mutually nonantagonistic phytopathogenic fungi. After a period of incubation, organisms that were antagonistic to the four phytopathogens, as indicated by zones of inhibition, were isolated. The degree of antagonism exerted by

these organisms on 10 phytopathogens was assessed in tests in which individual phytopathogens were sprayed on agar plates that had been streaked four days previously with a single antagonistic organism. The antagonists that were the most inhibitory were grown in five liquid media. The stability of the antibiotics in culture centrifugates was assessed with respect to pH, heat, and storage. Out of approximately 70,000 colonies of soil microorganisms, an isolate of *Bacillus subtilis* and five *Streptomyces* isolates were selected for further work.

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THE INFLUENCE OF NITROGENOUS COMPOUNDS ON THE GROWTH OF HELMINTHOSPORIUM GRAMINEUM IN CULTURE[1]

RICHARD H. CONVERSE[2]

(WITH 4 FIGURES)

Helminthosporium gramineum Rabh., the causal organism of the stripe disease of barley, exhibits a high level of parasitism. The fungus resembles the seedling-infecting smuts in many ways. It attacks only young seedlings, invading their shoots systemically and evoking severe symptoms in the host leaves as they mature. The existence of physiologic races of the pathogen has been reported by several workers (**1**, **4**, **9**, **10**). Barley varieties differ in their levels of resistance to this disease (**8**, **11**).

Because *H. gramineum* possesses many of the attributes of a specialized facultative saprophyte yet grows readily on synthetic culture media, it was used for a series of investigations on the effect of barley plant products and of nitrogenous compounds on mycelial growth.

Preliminary experiments indicated that the addition of expressed barley plant sap to a glucose salts basal medium quadrupled the growth of the fungus. There were small differences between varieties in this respect, but no consistent trend was found. Substances diffusing from barley germinating on a glucose salts agar increased average colony diameters of the fungus 6–8% as compared to comparable controls. These differences were small but

[1] A portion of a thesis presented to the Faculty of the Graduate School of the University of California January, 1951 in partial fulfillment of the requirements for the degree Doctor of Philosophy. The author acknowledges the aid and advice of several members of the Division of Plant Pathology of the University of California and particularly the help of Dr. B. R. Houston under whose guidance this work was done.

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highly significant statistically when an analysis of variance was performed, and paralleled the differences in stripe disease susceptibility of the two barley varieties tested.

The studies to be reported were designed to attempt duplication in chemically defined media of the stimulation observed by these barley products.

MATERIALS AND METHODS

Single spore isolates or lines of *H. gramineum* of known history were used throughout this study. Isolates A and B were recovered from naturally infected barley in California, while isolate C, a stable strain of the fungus found to be pathogenic to two susceptible California barley varieties, was obtained from the University of Wisconsin. Isolate A was used during the first part of these investigations. It was found to sector so frequently that isolate C was substituted for later nutrient studies. Where comparisons between two isolates were desired, isolate B was used.

Stock cultures were maintained on potato glucose agar. The primary inoculum for all nutrient studies consisted of small uniform agar discs from advancing margins of week-old stock cultures grown in petri dishes.

The following basic medium, found by Houston [3] to be the best carbon-salts combination for growth of this fungus, was used for all experiments:

Glucose	40 g.
Nitrogen source to furnish	0.245 g.
KH_2PO_4	0.30 g.
K_2HPO_4	1.20 g.
$MgSO_4 \cdot 7H_2O$	0.30 g.
$FeCl_3 \cdot 6H_2O$	1 ppm
$ZnSO_4 \cdot 7H_2O$	1 ppm
$CuSO_4 \cdot 5H_2O$	1 ppm
$MnSO_4 \cdot 5H_2O$	1 ppm
Distilled water	1000 ml.

This medium, using an amount of NH_4NO_3 (0.70 g./l.) known to furnish twice the optimum nitrogen requirement for the fungus, was used as the control and will hereafter be referred to as the basal medium.

[3] Unpublished information from Dr. B. R. Houston, Division of Plant Pathology, University of California, Davis, California.

When growth was to be measured in nutrient solutions, these solutions were portioned in 50 ml. amounts into 250 ml. Erlenmeyer flasks.

Each replicate was handled identically. An inoculum disc of standard diameter was aseptically placed on the bent end of a glass rod which passed through the cotton plug of the Erlenmeyer flask containing the nutrient solution. The disc was allowed to touch the solution. The flask was placed in the dark at $25 \pm 1°$ C for the required period with a minimum of disturbance. The resulting compact mycelial mat was washed repeatedly, air dried to a constant weight, and weighed. The average dry weight of mycelium was taken as the measure of growth for all media. Average values were usually based on five replicates. In general variation between replicates of a treatment in a given experiment was moderate, while differences between isolates and within a given isolate over time were often quite marked.

The pH of the residual nutrient solution was determined by means of a Beckman pH meter, Model G. If the culture medium remaining was to be analyzed for nitrate or ammonium content, it was reautoclaved and stored under refrigeration until used. Nitrate was determined colorimetrically by the technique of Blom and Treschow (3) as modified by the late Dr. W. O. Williams of the University of California. Ammonia determinations were made according to the A.O.A.C. method 12.58 (2) as modified by the Division of Agronomy, University of California.[4]

EXPERIMENTAL RESULTS

Inorganic nitrogen preferences. Ammonium nitrate was added to the basal medium as the nitrogen source for *H. gramineum*. The rate of preferential utilization of ammonia could be followed by noting the progressive pH changes of the medium. The ammonium and nitrate concentrations and observed pH values of media incubated with isolate C for various lengths of time are presented in FIG. 1. The pH-time curve follows the drop-and-rise

[4] The writer is indebted to Mr. Douglas Stuart of the Division of Agronomy, University of California, Davis, California for assistance in making the ammonia determinations.

pattern expected for a fungus that utilizes ammonia in preference to nitrate during the early period of growth. A theoretical pH for each lot of medium was calculated from the concentration of ammonia and nitrate left in the medium by each mycelial pad at a given time and a knowledge of the buffer capacity of the medium.

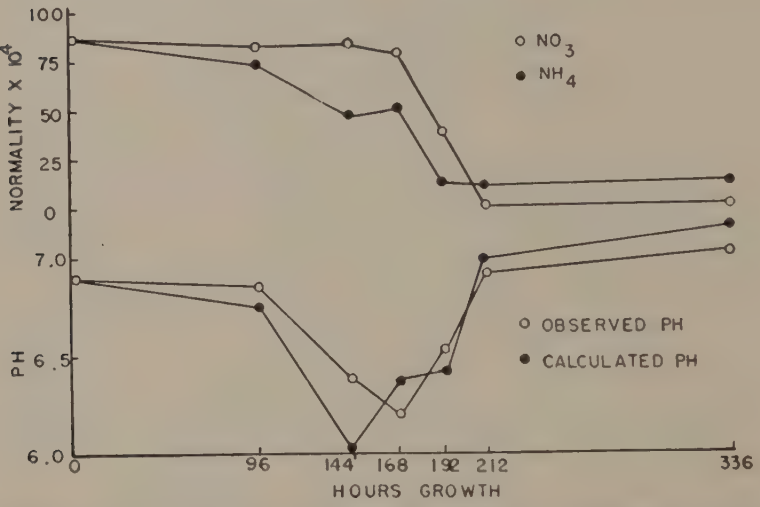

FIG. 1. Changes in average ammonia and nitrate concentrations and observed and theoretical pH values of media residues of individual cultures when incubated with isolate C of *H. graminicum* at intervals up to 336 hours at 25° C.

These theoretical pH values, plotted in FIG. 1, follow the general pattern of the pH values originally determined for the solutions. Comparable curves for isolate B are similar. By the "t" test for paired items the fit between observed and calculated pH values in FIG. 1 is poor and would occur only one time in one hundred by chance. However, errors introduced by autoclaving spent media may account for much of this disparity, and the changes in nitrate and ammonium nitrogen calculated for spent media are assumed to represent utilization trends of the fungus. Only 10% of available nitrate was used during the first 150 hours of growth, while 50% of the ammonia was taken up from the medium in the case of both isolates. At 225 hours nitrate removal became complete for both isolates, while ammonia removal approached 100% asymptotically.

The pH-time curves of isolates B and C resembled each other. Similar curves for isolate A were of the same general shape except that the magnitude of the pH drop and the rate of subsequent rise were much greater, values as low as pH 3.5 being obtained at 168 hours, followed by a change to pH 6.4 in 24 hours. Determination of nitrate and ammonia levels in media incubated with isolate A were not made because of its instability in culture.

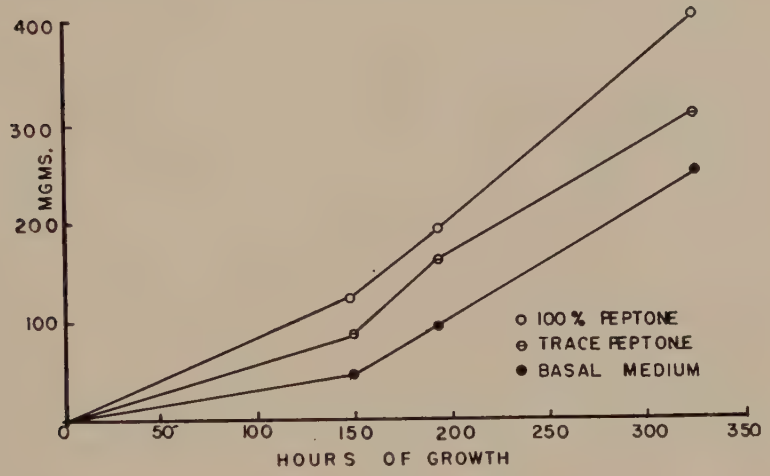

FIG. 2. Average dry mycelial weights of isolate A grown in peptone medium, trace peptone medium, and basal medium at 25° C over a series of time intervals.

Amino acid preferences. The response of the fungus to the addition of peptone to the basal medium is shown in FIG. 2. When Difco Bacto Peptone was used to furnish 100% or 5% of the total nitrogen of the medium, marked increases of growth of isolate A occurred as compared to that in basal medium. Similar but less marked stimulation occurred when isolate C was used.

The vitamins biotin, riboflavin, thiamin, pantothenic acid, inositol, nicotinic acid and pyridoxine, known to be present in the peptone, were added singly and in combination to the basal medium. Pyridoxine was supplied at the rate of 125 mmg./l. and nicotinic acid at 100 mmg./l.; all other vitamins at 10 mmg./l. In all instances these vitamins failed to increase the growth of isolate C over that

obtained in basal medium alone. Elliott (5) also failed to find vitamins of the B complex of value to this fungus.

Amino acids were used singly as nitrogen sources. In each case the amino acids furnished half of the nitrogen supply of the medium, the rest being supplied by ammonium nitrate. The ammonium nitrate present in the amino acid media represented a near-optimum supply of inorganic nitrogen for this fungus. Therefore any stimulatory or depressing effect of the added organic

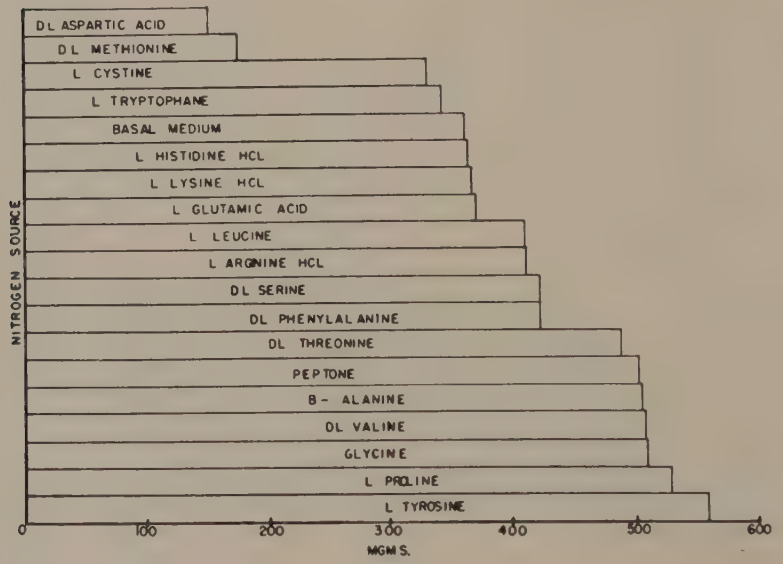

FIG. 3. Mycelial weight responses of isolate C of *Helminthosporium gramineum* to various amino acid media after 336 hours growth at 25° C.

nitrogen could be compared directly with the effect of the excess supply of inorganic nitrogen in the basal medium. FIG. 3 presents the average mycelial weights of isolate C at 336 hours obtained using various amino acids as compared to peptone and to ammonium nitrate as nitrogen sources.

Six distinct mycelial weight groups were obtained with these amino acid media when the data were subjected to an analysis of variance. The media falling in a given weight group produced statistically homogeneous growth responses. There were statisti-

cally significant differences between weight groups. The weight groups were: I, DL aspartic acid and DL methionine; II, L cystine and L tryptophane; III, basal medium, L histidine HCl, L lysine HCl, and D glutamic acid; IV, L leucine, L arginine HCl, DL serine, DL phenylalanine; V, LD threonine, peptone, β-alanine, DL valine, glycine; and VI, L proline and L tyrosine. It will be noted that six of the amino acids supported a growth as great or

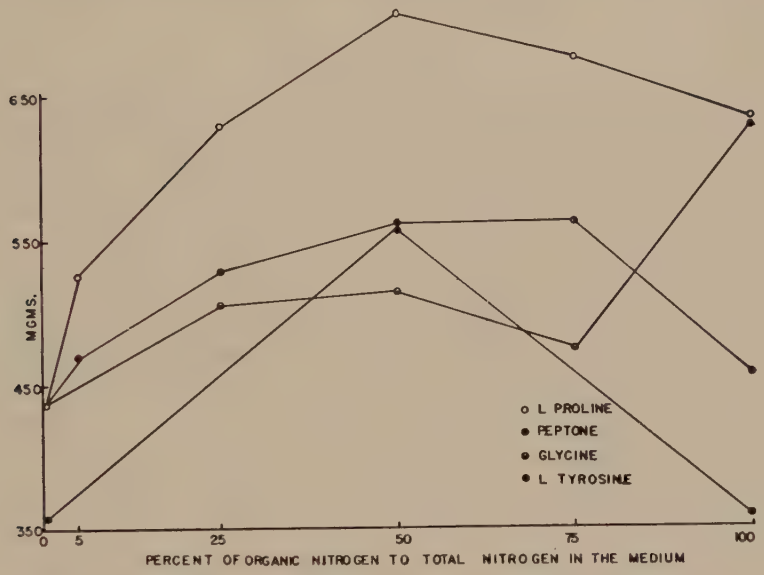

FIG. 4. The effect of changing the per cent of amino acid nitrogen supplied in media, total nitrogen being constant for each medium, on the average dry weight of mycelium of isolate C in milligrams after 336 hours of growth at 25° C.

greater than that supported by peptone. The stimulation by these amino acids and by peptone approached the growth response evoked by barley plant extracts.

Not only did amino acids differ greatly among themselves as to their ability to stimulate the growth of the barley stripe fungus, but the proportion of total nitrogen furnished by a given amino acid had marked influence on its stimulatory effect. In FIG. 4 the effect on growth of isolate C of increasing the per cent of organic-nitrogen in the medium while keeping total nitrogen constant is

illustrated. With the exception of the aberrant response of the 100% glycine nitrogen medium, peptone and the amino acids proline and glycine were most stimulatory when they supplied half the medium nitrogen. The tyrosine curve of FIG. 4 follows this same pattern. However, the data for this amino acid were obtained separately from those for the other materials and are not directly comparable, as the weights of mycelium formed in the basal medium controls were abnormally high in this latter experiment.

DISCUSSION

The barley stripe disease fungus was found to make some growth on all of the nitrogen sources tested. None of the B vitamins used stimulated growth over comparable basal medium checks. The fungus belongs among the *Helminthosporium* species reported by Lilly and Barnett (7) as able to utilize nitrate nitrogen. Ammonia and amino nitrogen were also utilized. However, the growth promoting ability and preferential use of all these nitrogen sources were not the same. No attempt will be made to explain the physiological significance of the mycelial weight groups found when comparing amino acids.

If the quantitative amount of mycelial growth supported by a given nutrient is assumed to reflect its nutritional value to the fungus and to the survival of the fungus in nature, then certain amino acids are of much greater nutritional value than inorganic nitrogen or other amino acids. For example, L tyrosine stimulated growth (of isolate C) increasing mycelial weight over controls up to 150%. Using other isolates (isolates A and B, respectively) the author found barley extracts to give 400% of control weights and products diffusing from germinating barley to give 108% of control weights.

Hrushovetz (6) has stated that *Helminthosporium sativum* root rot affects the concentration of amino acids in wheat roots. The pathogenicity of *H. sativum* is influenced by the kinds of amino acids supplied to the inoculum in culture. Wain and Wilkinson (12) found that organic solutes including amino acids diffused from germinating pea seeds into the environment. The relation of the nutrition and parasitism of fungi is a highly speculative field.

It is possible that the amino acid status of barley seedlings may exert some influence on the susceptibility of the seedlings to the stripe disease. Because no studies were undertaken of the amino acid status of seedlings of the various barley varieties used, the data here reported are admittedly inconclusive as to the role played by the amino acid status of the host in determining the inception of the barley stripe disease. The data do indicate a possible relationship.

SUMMARY

Helminthosporium gramineum Rabh. exhibited a marked growth response in culture to barley plant extracts and to products diffusing from germinating barley. The organism utilized the ammonium ion in preference to nitrate ion in early stages of growth. Peptone was found to stimulate growth. The B vitamins present in peptone were not found to increase fungus growth when added to basal medium. Growth in certain amino acids plus ammonium nitrate was much greater than that in ammonium nitrate as the sole nitrogen source. In the presence of L tyrosine, L proline, glycine, DL valine, β-alanine, or DL threonine, fungus growth equalled or exceeded that in peptone and approached that obtained with barley plant extracts. Amino acids were the only nutrients tested that gave this type of response. The possibility of a relationship between the response of *H. gramineum* to amino acids in culture and the establishment of the fungus in the host is suggested.

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OBSERVATIONS ON STREPTOMYCES GRISEUS. V. SYNTHETIC MEDIA FOR VITAMIN B_{12} PRODUCTION WITH SPECIAL REFERENCE TO POSSIBLE VITAMIN B_{12} PRECURSORS

EUGENE L. DULANEY AND PATRIC L. WILLIAMS

Since the original isolation of vitamin B_{12} from liver (19), a number of papers have reported the isolation of the same vitamin from microbial metabolism solutions, thus indicating a practical source of the compound (13, 14, 15, 16, 18, 23). This capacity for vitamin B_{12} production appears to be widespread in the genus *Streptomyces,* including the antibiotic-producing species.

One of the primary problems that must be overcome in putting a new microbial product into production is that of devising a suitable medium. Such media are usually composed of complex organic materials that have the two advantages of being inexpensive and of supporting high-broth potencies of the compound in question. These complex organic media, however, are frequently not useful as tools in the study of various problems that arise, and it becomes necessary to develop simple synthetic media to aid in the elucidation of these problems. The present paper treats of the development of some simple synthetic media for use in searching for possible vitamin B_{12} precursors and in studying the relationship between growth and vitamin B_{12} production.

METHODS

The basal medium used in these studies was a modification of the glucose-inorganic salts medium used previously in our studies on synthetic media for streptomycin production (8, 9). Preliminary experiments indicated that maximum yields of vitamin B_{12} were obtained when the glucose and $(NH_4)_2HPO_4$ levels were raised and Co^{++} as $Co(NO_3)_2 \cdot 6H_2O$ was added. The composition

finally decided on was glucose 20.0 gm, $(NH_4)_2HPO_4$ 7.5 gm, NaCl 5.0 gm, K_2HPO_4 2.0 gm, $MgSO_4 \cdot 7H_2O$ 1.0 gm, $CaCl_2$ 0.4 gm, $FeSO_4 \cdot 7H_2O$ 10.0 mg, $ZnSO_4 \cdot 7H_2O$ 10.0 mg, $Co(NO_3)_2 \cdot 6H_2O$ 10.0 mg, and distilled water to one liter. This level of Co^{++}, 2γ/ml, is in excess of the amount required for maximum vitamin B_{12} production but is well below the inhibitory level.

This medium is prepared by dissolving the salts in two-thirds of the water and the glucose in one-third of the water and combining them aseptically after autoclaving at 15 lbs pressure for 16 minutes. When possible the compounds being tested as precursors were added aseptically to the medium after sterilization in one ml. amounts so that the final desired concentration was obtained. The pH of the medium, even after supplementation with the adjuvants, was never below 6.5 nor above 7.3.

The strain of *Streptomyces griseus* used in these experiments is a mutant that has descended from the strain used previously in our studies on streptomycin production (**8**, **9**) and is strain G of paper IV of these series (**10**). Spores of this strain, lyophilyzed in sterile soil, served as the stock from which all cultures used in these experiments descended. Spores in soil were spread over slants of a nutrient agar medium with a soybean meal base. After incubation at 28° C for seven days, excellent spore crops had developed which were harvested by washing with sterile distilled water. One ml of this spore suspension was used to start each flask of vegetative inoculum. The vegetative inoculum was always developed in the glucose-salts medium, 30 ml/125 ml flask, on rotary shakers moving at 220 rpm and describing a circle one inch in diameter. After incubation for three or four days at 28° C, one ml of the vegetative growth that had developed was used to inoculate each fermentation flask. The fermentations, which were incubated under the same conditions as was the inoculum, were sampled at appropriate intervals, diluted in M/20 phosphate buffer, autoclaved at 15 lbs pressure for 15 minutes, and assayed by the cylinder plate method using Davis' *Escherichia coli* mutant as the test organism (**7**). The Dorner strain of *Lactobacillus lactis* was used as the test organism, however, when the medium contained methionine.

RESULTS AND DISCUSSION

Nitrogen sources for growth and vitamin B_{12} production. The glucose salts medium supports vitamin B_{12} broth potencies of approximately 3000 u./ml thus showing that *Streptomyces griseus* can synthesize the complex B_{12} molecule from glucose carbon and inorganic salts.

It appeared possible that increased yields could be obtained by the substitution of other carbon and nitrogen sources for the glucose and the $(NH_4)_2HPO_4$. Thus, some twenty-two nitrogen sources, principally single amino acids, were substituted for the $(NH_4)_2HPO_4$ at a level of 3.732 mg/N/ml. The compounds tested and the maximum results obtained are given in TABLE I.

TABLE I

NITROGEN SOURCES FOR GROWTH AND VITAMIN B_{12} PRODUCTION

Nitrogen source	Maximum vitamin B_{12} broth potency	Day of maximum
$(NH_4)_2HPO_4$	3,100	5
N-Z-amine	6,700	4
Urea	1,500	4
DL-α-Alanine	6,000	4
β-Alanine	8,300	7
L(+)Arginine HCl	1,000	7
Asparagine	5,400	4
DL-Aspartic acid	4,600	7
L(+)Cysteine HCl	3,000	7
L(-)Cystine	1,000	6
Creatine hydrate	no growth	
Creatinine	no growth	
L-Glutamic acid	3,000	6
Glycine	6,100	6
L-Histidine HCl	5,400	4
DL-Isoleucine	no growth	
L(-)Leucine	1,600	7
DL-Lysine HCl	1,400	7
DL-Methionine	700	6
L(-)Proline	15,000	6
DL-Serine	inhibition	
DL-Threonine	5,600	7
DL-Valine	no growth	

With one exception, the single amino acids, namely DL-alpha-alanine, Beta-alanine, glycine, L-histidine HCl, L(-)proline and L-arginine HCl, that had been found previously (8) to support relatively high streptomycin yields, also supported relatively good vitamin B_{12} yields. The one exception was arginine, and in this instance growth was slow and good cell weight were not obtained.

Had the experiment been prolonged past seven days, as was done in the previous test for streptomycin production, perhaps higher vitamin B_{12} broth potencies would have been realized. Two other amino acids, namely L-aspartic acid and DL-threonine, that had supported little if any streptomycin production, supported relatively good yields of vitamin B_{12}. DL-lysine HCl and DL-methionine supported somewhat lower yields of vitamin B_{12} and had been found previously to support little or no streptomycin production. Creatine, creatinine, DL-valine, and DL-isoleucine when used as single nitrogen sources, did not support growth.

There is a much closer correlation between growth and vitamin B_{12} production than there is between growth and streptomycin production. In all treatments where growth occurred, with one exception, some vitamin B_{12} was produced. The one possible exception was in the medium containing DL-serine. These metabolism solutions were inhibitory to the test organism, *Lactobacilus lactis*. It is still possible that these broths contained vitamin B_{12}. There is also an apparent correlation between the amount of growth obtained and the vitamin B_{12} level produced—a point that will be dealt with more fully later. The highest B_{12} broth potencies and the highest cell weights were obtained in the medium containing L(-)proline as the sole nitrogen source. Proline, as a single nitrogen source, had been found previously to support quite high streptomycin activities (8). It would appear that the series of mutant strains with which we have been working might well be termed "proline specific." The use of proline as a single nitrogen source has a marked effect on the physiology of these strains but not necessarily on other strains.

Carbon sources for growth and vitamin B_{12} production. The carbon sources used as substitutes for glucose were limited, with the exception of sucrose, to those that had been found previously (9) to support good growth of *Streptomyces griseus*. The possible substitutes were tested at a level of two per cent. The results of this test are given in TABLE II. None of these substitutes was particularly outstanding, although lactose, maltose, dextrin, starch, and mannitol supported yields that were significantly better than those obtained by the use of glucose. Perhaps in experiments

such as these, marked increases in broth potencies should not be expected. Practically nothing is known of the factors that limit vitamin B_{12} synthesis, and the mere substitution of single carbon and nitrogen sources may not be satisfying these limitations. Whatever these limitations are, later results have made it clear that the levels of carbon and nitrogen used in these experiments were above the optimum required for maximum growth and vitamin B_{12} production.

TABLE II

CARBON SOURCES FOR GROWTH AND VITAMIN B_{12} PRODUCTION

Carbon source	Maximum vitamin B_{12} broth potency	Day of maximum
Cellobiose	2600	3
Dextrin	4500	3
Galactose	3200	3
Glucose	3100	3
Glycerol	2400	3
Lactose	4500	6
Levulose	3100	6
Maltose	4700	6
Mannitol	3800	3
Mannose	3100	6
Starch	4200	3
Sucrose	900	3
l-Xylose	3400	6

Precursor studies. In a search for biological precursors, one is faced with a number of problems of which the primary one is that of the criteria to be used in judging as to whether or not a compound can serve as a precursor. The ultimate test for precursor activity would appear to be the use of "tagged" atoms in proving that the compound under test was assimilated into the molecule in question. This is impractical on a large scale, and we are faced with the necessity of using other methods. In these experiments, the preliminary basis for judging precursor activity was whether or not a compound, when added to a medium being metabolized by growing cells, resulted in an apparent increase in vitamin B_{12} broth potency. The weakness of this criterion is fully realized, and this point will be expanded somewhat later. It is within the bounds of this convention, however, that we speak of compounds that stimulate B_{12} production when an apparent increase in B_{12} broth potency is found in treatments to which these compounds are

added. Of course one expects an increase upon addition of a single compound only if the limiting reaction is being fed.

At the outset of our search for B_{12} precursors, very little was known about the structure of the vitamin B_{12} molecule. The presence of cobalt was known, and this was incorporated into our basic medium (**16, 20**). Under such conditions, our initial search for precursors became quite empirical. It involved the addition of complex organic plant and animal remains to the glucose-$(NH_4)_2HPO_4$-salts medium in the hope that a marked stimulation of broth potency would occur. This would then be followed by fractionation of the substance invoking the stimulation. The list of materials tested is long and need not be detailed but included fish meals, seed meals, yeast products, casein hydrolysates, distillery residues, etc. All of these materials were tested at a level of 5 mg/ml. In no instance was there an increase in broth potency that could not be attributed to an increase in cell weight.

Following this unsuccessful test of the above materials, a number of biologically active compounds, namely amino acids, organic acids, purines, and pyrimidines, were screened as possible precursors. All of the amino acids listed in TABLE I were added singly at a level of 0.46 mg/N/ml. The carboxylic acids, *i.e.*, fumarate, malate, pyruvate, acetate, citrate, lactate, succinate, and ethloxalacetate were used at a level of 5 mg ml, whereas adenine, guanine, xanthine, uracil, orotic acid, cytosine, thymine, and yeast nucleic acid were tested at a level of 1 mg/ml. Detailed results of these experiments need not be given, but it will suffice to report that the use of none of these compounds resulted in precursor-like activity. In several instances, significant increases in broth potencies were obtained. Here again, however, these increases were not marked and could be attributed directly to stimulation of growth.

All of the above materials were tested at a time when little was known about the structure of the B_{12} molecule. In retrospect, most of the materials should not have been expected to show precursor activity, but some of them would have been selected for testing on the basis of their structures.

With increase in knowledge of the structure of the B_{12} molecule, we are able to test for precursor activity compounds whose formulations occur in the molecule. Here we are on much firmer ground in our search for precursors.

At the present time the vitamin B_{12} molecule is known to contain cobalt (**20**), 1-amino-2-propanol (**25**), α-ribazole phosphate (1-α-D-ribofuranosido-5,6-dimethyl benzimidazole phosphate) (**2, 3, 4**), and an unknown portion which has been suggested as being a pyrrole or porphyrin-like (**1**).

All of the portions of the B_{12} molecule, *i.e.,* amino propanol, α-ribazole and the unknown moiety, have been tested singly, at a level of 100 γ/ml and in all possible combinations. In no instance was any increase in B_{12} broth potency noted. The failure of amino-propanol to stimulate B_{12} production was followed by a test of L-threonine and hydroxyglutamic acid. Amino-propanol could be formed by the decarboxylation of threonine and hydroxyglutamic acid could be decarboxylation go to threonine and thence to amino-propanol. The L-threonine and hydroxyglutamic acid, as well as N-acetyl hydroxyglutamic acid, were tested singly at levels of 100 γ/ml and in combination with the other parts of the molecule. The results did not indicate any stimulation of B_{12} production.

The compounds tested as possible precursors for the unknown part of the molecule were selected on the basis that this unknown moiety is a pyrrole or porphyrin-like. The unknown moiety, singly at a level of 100 γ/ml or in combination with α-ribazole and/or amino-propanol, did not increase the B_{12} broth potency. Pyrrole and L(-)proline likewise elicited no response when tested singly or with the other fragments of the molecule. Bilirubin, chlorophyll, cobalt protoporphyrin or cobalt hematoporphyrin, singly at a level of 100 γ/ml or in combination with α-ribazole and/or amino-propanol were inactive as precursors. It should also be noted that glycine, which has been shown to be a precursor of porphyrins (**22**), was also inactive in increasing B_{12} broth level.

Following our failure to find precursors for the amino-propanol and unknown part of the molecule, our attention was turned to the α-ribazole phosphate moiety. The α-ribazole singly, at several

concentrations up to 100 γ/ml or in combination with amino propanol or with the unknown fraction did not induce a rise in the level of B_{12} produced. This was likewise true of 5.6-dimenthylbenzimidazole and 2,3-diamino-5,6-dimethyl benzene. As was expected 2,3-diamino-5,6-dichlorobenzene was quite inhibitory. o-Phenylenediamine (diamino benzene), when used singly, induced a marked increase in the apparent level of B_{12} in the broth. Likewise o-nitroaniline, o-xylidine, and 3,4-diaminotoluene, when used singly, stimulated B_{12} production. However, the use of benzene, aniline, or nitrobenzene did not result in a stimulation.

p-Aminobenzoic acid, reported by Davis (**6**) to be involved in B_{12} synthesis by *Escherichia coli,* was inactive in stimulating B_{12} production by *Streptomyces griseus.* This was likewise true of benzoic acid, quinic acid, and shikimic acid, the last of which has been reported to be an important intermediate in the synthesis of cyclic compounds (**5**). Other benzene derivatives, *i.e.,* sodium hydroxybenzoate, saccharine, p-dimethylaminobenzaldehyde, benzlycarbinol, and sulfanilic acid, were inactive in stimulating B_{12} production.

In summary, of all of the compounds tested, only o-phenylenediamine, o-nitroaniline, o-xylidine, and 3,4-diaminotoluene have invoked a marked increase in B_{12} broth potency. The relationship between the structure of these compounds and the B_{12} molecule is obvious. However, α-ribazole, 5,6-dimethylbenzimidazole, and 2,3-diamino-5,6-dimethylbenzene are structurally closer to the B_{12} molecule than are any of the stimulatory compounds. Moreover, these three compounds show vitamin B_{12} activity in the rat (**11, 12**). Wooley has presented evidence (**26, 27**) that the diaminodimethylbenzene increased both the B_{12} and B_2 production by *Bacillus megatherium.* This prompted us to test a series of possible riboflavin inhibitors in the hope that a shift to B_{12} synthesis would follow. These compounds, tested at a level of 25 γ/ml, included 2-desoxy-2-imino-riboflavin, isoriboflavin, phosphorylated isoriboflavin, galactoflavin, 6,7-dihydro-9 (I L-lyxityl) isoalloxazine lyxoflavin, and ethyl-2-keto-3-carboxy-5,6 (4,5-dimethylbenzo) N-ribityl pyrazine. None stimulated vitamin B_{12} production.

As previously noted, the fact that the addition of a compound to inoculated medium results in an increase in B_{12} content over that in the unsupplemented broth does not conclusively prove that this adjuvant is acting as a precursor, even if this increase is a marked one. Indeed this does not even prove that there has been an increase in the amount of the B_{12} produced. *A priori* this apparent stimulation can be attributed to the stimulating compounds acting in one or more of the following ways, namely, a) by increasing the amount of growth, b) by having a sparing action of the B_{12}, thus increasing the response of the assay organism to the level of B_{12} produced, c) by educing a growth response in the assay organism, d) by decreasing the rate of B_{12} destruction, e) by acting as a precursor, and f) by acting as a competitive inhibitor.

It can be proved experimentally that addition of these compounds does not bring about a marked increase in growth. This is particularly pertinent because in these systems growth and B_{12} production are closely correlated. It can also be proved that the compounds do not invoke a growth response with the assay organism. The possibility that these stimulating compounds were able to exert a sparing action on B_{12} was a distinct one in view of the report by Hendlin and Soars (**17**) that o-phenylenediamine, o-xylene, and aniline had a B_{12}-sparing action with *Lactobacillus lactis*. Our results, however, showed no such sparing action with *Escherichia coli*.

The mode of action of these compounds could possibly lie in their inhibition of B_{12} destruction. It must be understood that the assay level of a fermentation broth represents the amount of product in the broth at time of assay, that is the amount of product produced in excess of the amount that is destroyed. Any decrease in the rate of destruction will result in an increase in the broth level. Some light could be thrown on this point if *Streptomyces griseus* could be shown to actively destroy or not to destroy B_{12}. It can be demonstrated experimentally that there is not a marked decrease in the level of B_{12} added to growing cells of *S. griseus*. This, however, is not conclusive proof that the added vitamin is not being destroyed. A non-producing mutant of *S. griseus* does not destroy B_{12}, but here there is the possibility that the capacity

for destruction of B_{12} was lost at the time that the ability to synthesize the compound was lost. This possible mode of action then is difficult to evaluate. Our data would appear to indicate, however, that the rate of B_{12} destruction, if any, is not great enough to account for the marked and rapid increase in B_{12} even if this destruction was completely terminated.

The ultimate proof of precursor activity lies in determining whether labeled compounds are incorporated into the molecule in question. This method was applied by the use of 1,4,5,6-deutero-o-phenylenediamine. Assuming the compound was incorporated into the B_{12} molecule, the deuterium in the 5 and 6 positions would be replaced by methyls. It would also appear to be possible that the o-phenylene diamine could be incorporated into the molecule without being methylated with the resultant formation of a compound different from vitamin B_{12}. The results, however, make it obvious that the o-phenylenediamine, if it is being incorporated into the B_{12} molecule, is being introduced into no more than 20% of the vitamin B_{12} produced (24). This certainly does not explain the remarkable enhancing activity of the compound. Hence, a mode of action other than that of a precursor must be sought.

The most probable route of action of the stimulatory compounds lies in their being competitive inhibitors. Competitive inhibition has many permutations, however, and the exact mode of action is obscure. All of the stimulatory compounds are most effective at a level at which they are slightly inhibitory. For example, o-phenylenediamine is effective at a level of 50 γ/ml but is most effective at 100–200 γ/ml or the level at which growth at first is retarded somewhat, then the inhibition is overcome. Under such conditions, use of o-phenylenediamine at a level of 100 γ/ml results in an increase in B_{12} broth potency of 100% (3000 u./ml) and sometimes more. Increases of 200% have been obtained. Additions of o-phenylenediamine to three-day-old fermentations, when growth is near a maximum, has no inhibitory effect and little or no effect on B_{12} production. o-Xylidine and 3,4-diaminotoluene are also most active at a concentration of approximately 100 γ/ml o-Nitroaniline, however, is much more inhibitory and

cannot be used at a level much above 10 γ/ml. These latter three compounds, however, are only approximately 40% as effective as o-phenylenediamine in increasing B_{12} broth potency.

It would be enlightening if the inhibitory and stimulatory actions of the compounds could be separated. The inhibition by o-phenylenediamine may be overcome by the organism in several ways. An enzymatic adaption could be involved. Likewise a compound (B_{12} ?) antagonistic to o-phenylenediamine could be produced by the growing cells. Certainly higher levels of o-phenylenediamine are tolerated if vitamin B_{12} is added at the start of the fermentation.

The o-phenylenediamine may be acting as a selective agent, allowing the resistant nuclei to develop. If this is true, resistant nuclei would have to be very numerous and the mutation to resistance quite common to explain the rapid reversal of inhibition. In addition attempts to select for resistance to o-phenylenediamine met with little or no success though these attempts were not extensive. Such resistant strains, if available, would aid in determining if increased resistance is accompanied by increasing response to increasingly higher levels of o-phenylenediamine.

The response to o-phenylenediamine is not limited to the strains of *S. griseus* with which we have been working nor is it found in all other species of *Streptomyces*. Of some 13 different vitamin B_{12}-producing *Streptomyces* species tested, three gave a marked response to o-phenylenediamine.

Growth and vitamin B_{12} production. It has been noted above that there is an apparent relationship between growth and vitamin B_{12} production. This can be demonstrated by the use of a simple synthetic medium containing a single sugar, such as glucose or maltose, in combination with a single nitrogen source such as $(NH_4)_2HPO_4$, proline, histidine, alanine, or glycine. If one uses an excess of sugar and varies the level of nitrogen from a very low concentration of about 0.2 mg/N/ml to an excess of that required for maximum growth, the correlation between growth and B_{12} production becomes apparent.

As is to be expected, the dry-cell weight increases with increasing nitrogen level until a maximum cell weight is obtained. The

vitamin B_{12} level also increases with increasing nitrogen level and with increasing cell weight, the maximum B_{12} level occurring at the time of maximum cell weight. Addition of nitrogen in excess of that required for maximum cell weight does not result in increased production of B_{12} but may bring about lower cell weights with a corresponding decrease in vitamin B_{12} production.

The ratio of B_{12} produced to that of the dry-cell weight does not change appreciably, however, with changing nitrogen level of the medium. Indeed the highest production of B_{12} per mg dry weight of cells usually occurs in the medium with the very low nitrogen levels.

SUMMARY

Streptomyces griseus is capable of producing vitamin B_{12} in a simple synthetic medium containing glucose, $(NH_4)_2HPO_4$ and other inorganic salts. Alanine, glycine, histidine, and proline can be substituted, singly, for $(NH_4)_2HPO_4$. Lactose, maltose, dextrin, starch, and mannitol are suitable as substitutes for glucose.

A large number of complex organic materials, amino acids, carboxylic acids, purines, pyrimidines, and degradation products of vitamin B_{12} were tested for stimulation of vitamin B_{12} production. None induced a stimulation.

Ortho-phenylenediamine, ortho-nitroaniline, ortho-xylidine, and 3,4-diaminotoluene when used as single substituents invoked a significant increase in vitamin B_{12} broth potency.

The most active compound, ortho-phenylenediamine is not a precursor. It possibly acts as a competitive inhibitor.

There is a close correlation between growth and vitamin B_{12} production.

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THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON THE COURSE OF SYSTEMIC MONILIASIS IN MICE[1]

George H. Scherr

(with 1 figure)

It had become evident to the author that mice infected with moniliasis during the hot summer months displayed a much more severe infection and higher death rate than those infected under more moderate climatic conditions. The following experiments performed under controlled temperature conditions demonstrate the significant role of environmental temperature in the pathogenesis of moniliasis for mice.

MATERIALS AND METHODS

The mice used were a white Swiss strain, departmental stock. All animals received tap water and Purina chow pellets *ad libitum* under both normal and experimental conditions.

Candida albicans strain *171D* has been described in a previous paper (3).

The medium used was a broth employed by Reilly, Harris, and Waksman (2). Its composition is glucose, 1.0%; peptone, 0.5%; beef extract, 0.5%; NaCl, 0.5%; distilled water. Thirty ml of broth contained in a 125 ml Erlenmeyer was loop-inoculated with *C. albicans 171D* and incubated at 30° C. The centrifuged pellet from such a 48-hour culture was suspended in 16 ml. of sterile saline. This final cell suspension contained 1.1×10^9 cells/ml as determined by plate count.

[1] The work reported here was originally performed by the author at the Department of Bacteriology of the University of Kentucky. The data confirming and extending this temperature effect has since been done as part of a study supported by a contract between the Office of Naval Research, Department of the Navy, and the Creighton University School of Medicine.

Cages containing 10 male mice each were placed in incubators whose temperature ranges were 5°–12° C, 15°–20° C, 28°–32° C, and 35°–37° C. Each mouse received a single infecting dose of 0.2 ml of the *Candida* cell suspension intraperitoneally 24 hours after being placed in the incubator. Uninfected controls were similarly incubated. At the end of 30 days all surviving animals were autopsied.

The degree of dissemination of moniliasis was gauged from the number, size, and distribution of the lesions. As a rule the condition of the kidneys is an excellent indicator of the severity of the infection. The degree of dissemination was assigned a numerical value, as follows:

0.....no visible pathology
1.....few, isolated lesions
2.....frequent lesions
3.....many lesions
4.....marked dissemination of lesions
5.....very marked dissemination

If the animal died, presumably as a result of *Candida* dissemination, prior to the end of the exposure period, a value 1 was added to the base value 5 for every day intervening between death and the termination of the experiment. For example, if an animal died 3 days before the experiment culminated, the dissemination value was designated by the number 8. Thus, the dissemination values recorded here are a measure of both severity of infection and mortality rate. No dissemination values are given for those animals which were found mutilated or were killed accidentally; they are represented by a dash (–) in the various tables.

RESULTS

The data for this experiment are compiled in TABLE I and graphically illustrated in FIG. 1. The temperature ranges of 15°–20° C and 28°–32° C showed the lowest mean dissemination values, 14.2 and 13.8, respectively. A cold temperature (5°–12° C) enhanced the severity of infection manifest at moderate temperatures. The validity of the difference between the mean dissemination values found at cold and moderate temperatures was supported by the statistical analysis of *t*. An environmental temperature of 35°–37°

TABLE I

THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON MICE SYSTEMICALLY INFECTED WITH *Candida albicans 171D*

(Duration of experiment—30 days)

Incubation temp. (°C)	Dissemination values	Infected mice		Dissemination values Controls uninfected
	3	Mean	24.3	—
	16	Av. dev. from mean	7.2	0
	18	Stand. dev. from mean	8.7	0
	27			0
5–12	27			0
	30			0
	30			0
	30			0
	31			0
	31			0
	3	Mean	14.2	
	3	Av. dev. from mean	11.8	0
	4	Stand. dev. from mean	12.3	0
	4			0
15–20	5	*t* for 15°–20° compared to		0
	7	5° C	2.0	0
	26	Degrees of freedom	18.0	0
	27	P	.025–.05	0
	30			0
	33			0
	2	Mean	13.8	0
	3	Av. dev. from mean	11.0	0
	3	Stand. dev. from mean	12.0	0
	3			0
28–32	3			0
	16			0
	19			0
	24			0
	32			0
	33			0
	27	Mean	28.2	—
	27	Av. dev. from mean	1.7	0
	27	Stand. dev. from mean	1.8	0
	27			0
35–37	27	*t* for 15°–20° C compared to		0
	27	35°–37° C	3.38	0
	27	Degrees of freedom	18	0
	31	P	<.005	0
	31			0
	31			0

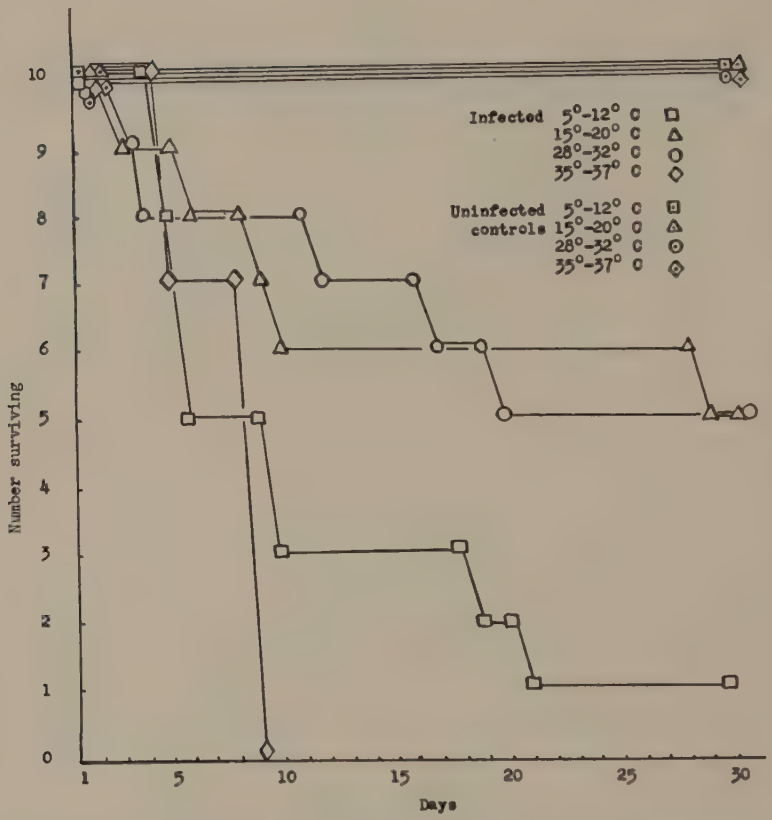

FIG. 1. The effect of environmental temperature on mice systemically infected with *Candida albicans 171D*

C was more detrimental to these infected mice than any of the other temperature ranges studied. In marked contrast to the 50% mortality found at both moderate temperature ranges after 30 days, 100% of the infected animals kept at 35°-37° C were dead after only 9 days.

Uninfected controls kept at the same temperatures as the experimental groups of mice showed no untoward effects.

DISCUSSION

This temperature effect for moniliasis has been confirmed by other experiments. In addition, it has been possible to correlate

sudden peaks of high mortality among *Candida*-infected mice in our colonies with peaks of high temperature occurring during the course of the summer. This enhancement of moniliasis by high environmental temperatures complicates experimental work still further if studies are being conducted with such compounds as cortisone, whose toxicity for mice is also markedly affected by changes in environmental temperature (unpublished data of author).

Kuhn (1) found that Swiss mice infected with *Cryptococcus neoformans* that were kept at 35°–36° C showed a higher rate of survival than controls kept at 24°–27° C. Data for other deep mycotic infections with respect to this temperature effect might profitably be collected and assessed as a potential factor affecting the pathogenesis of these diseases. A clinical application of this temperature effect is conceivable but must be preceded by additional laboratory work.

SUMMARY

Swiss mice systemically infected with moniliasis showed a more severe dissemination of the infection and higher death rate when kept at controlled temperatures of 5°–12° C and 35°–37° C than at temperatures of 15°–20° C and 28°–32 C. Uninfected animals kept at the same temperatures as the experimental animals were apparently unaffected. The significance of increases of environmental temperature on the pathogenesis of moniliasis is discussed.

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NOTES ON THE GENUS CAMPTOMERIS, FUNGI IMPERFECTI

ERNST A. BESSEY

(WITH 34 FIGURES)

In 1927, H. Sydow described under the name *Camptomeris calliandrae* Sydow a new genus and species of dematiaceous Fungi Imperfecti, collected by him in Costa Rica on the living leaflets of *Calliandra similis* Sprague and Riley (Family Mimosaceae). Three years later the same author gave the name *Camptomeris leucaenae* (Stevens and Dalbey) Sydow to a fungus collected by him in Venezuela upon the living leaflets of *Leucaena glauca* (L.) Bentham, also one of the Mimosaceae. He compared his fungus with specimens collected by F. L. Stevens in Puerto Rico on the same host and described (by Stevens and Dalbey, **1919**) as *Exosporium leucaenae* Stevens and Dalbey, and recognized that his own collections from Venezuela were identical with those of Stevens from Puerto Rico and belonged to his own previously described genus *Camptomeris*. In 1933 R. Ciferri described another species of this genus collected by him and Ekman in Santo Domingo upon *Desmanthus virgatus* (L.) Willdenow (also one of the Mimosaceae), calling it *Camptomeris desmanthi* Ciferri. Confusion was brought about when, a year later, Petrak (**1934**) redescribed this same fungus, collected in the same locality by Ekman, on the same host, calling it *C. desmanthi* (Ellis and Kellerman) Petrak. He based the change in the authority for the name on his claim that this is the same fungus that was described by Ellis and Kellerman (**1885**) from Kansas on *Desmanthus brachylobus* Bentham (now called *D. illinoensis* (Michx.) MacM.) as *Cercospora condensata* Ell. and Kell. var. *desmanthi* Ell. and Kell. This name was changed by Ellis and Everhart (**1887**) to *Cercospora desmanthi* (Ell. & Kell.) Ell. & Ev. The present author has studied the type of this latter species and has demonstrated that it is generically and specifi-

cally distinct from *Camptomeris desmanthi* Ciferri, of which typical material was also studied. In **1943** C. G. Hanford reported the occurrence of a fungus on *Albizzia coriaria* Welw. from Uganda, East Africa, under the name *Camptomeris albizziae* (Petch) Mason, its identity with *Helminthosporium albizziae* Petch, from Ceylon, having been noted on the herbarium sheet by E. W. Mason. In the herbarium of the Commonwealth Mycological Institute, in Kew, England, are specimens identified on the labels as belonging to *Camptomeris*, but not yet described under this name. Both are on species of *Acacia*, one from Jamaica and one from Natal, South Africa. In southern India, on *Albizzia lebbek* (Willd.) Benth., the same host as that of Petch's species from Ceylon, M. J. Thirumalachar collected a fungus which he published (**1950**) as *Helminthosporium albizzicolum* Thirumalachar and Narasimhan. Examination by the writer of type specimens reveals that this, too, probably should be called a species of *Camptomeris*. At Fort Myers, Florida, in December, 1950, the writer collected another unnamed species of this genus upon *Pithecellobium unguis-cati* (L.) Benth., for which the name *C. floridana* Bessey is to be proposed. Hansford (**1943**) described *C. tephrosiae* Hansf., on *Tephrosia candida* DC., from Uganda, but, as will be indicated further on, this does not seem to belong to *Camptomeris* as the writer delimits it. Except for this species, the hosts of *Camptomeris* all belong to the Mimosaceae and are all tropical or subtropical in their distribution. In 1951 Ciferri proposed the transferral of two other species of fungi to the genus *Camptomeris: Fusicladium robiniae* Shear on *Robinia pseudacacia* L. and *Septogloeum acaciae* Syd. on *Acacia arabica* Willd. In the fuller discussion of the species of *Camptomeris* will be given the writer's reasons for doubting the validity of these transfers.

The genus has been collected in Florida and Texas in the United States; and in Costa Rica in Central America; in Venezuela in South America; and in Jamaica, Puerto Rico and Santo Domingo, in the West Indies. In Asia it has been reported from India and Ceylon. It has been found in Africa at such widely separated localities as Sierra Leone, Gold Coast Colony, Uganda, and Natal. It has been described or named in herbaria under the generic names *Campto-*

meris, Exosporium, Helminthosporium, Cercospora, Cercosporidium, Passalora and *Stigmina*. It would appear, therefore, to be a worth while task to study the leaf-inhabiting species assigned to the foregoing genera insofar as they attack hosts belonging to the Mimosaceae.

All of the accepted species of *Camptomeris* are confined to the living leaves of mostly woody Mimosaceae where they form their conidiophores on the under side of the leaf. They do not usually produce strongly noticeable leaf spots. Mostly the upper surface of the leaf shows a slight yellowing, without a strongly colored margin. On the lower surface of the leaf, at distant intervals or closely placed, the fungus usually forms light brown sub-epidermal hypostromata of more or less isodiametric, rather cubical, fairly thin-walled cells. They may force their way through stomatal openings or, if large, may roll back the epidermis to permit the emergence of small, rather flat-topped sporodochia. In the more typical species of the genus (subgenus *Eucamptomeris*) the only cells emerging from the hypostroma are the enlarged, almost colorless, somewhat balloon-shaped megalophyses. From these exposed cells arise the usually dark-brown conidiophores which bear at or near their tips the single conidia. The conidiophores are more or less concentrically arranged, those toward the center being almost isodiametric and more upright, while those nearer the margin are flattened so as to be somewhat spatulate or even spoon-shaped. These marginal conidiophores are more outspread but then curve upward. The conidia, formed one to a conidiophore, are lighter brown in color and oblong in outline or obclavate or obpyriform or even lanceolate. The apical half may taper markedly or very slightly. The apex is rounded. The base is rounded or truncate, with a nearly central scar of attachment. The conidia have 2 to 3 transverse septa, rarely only 1 or 4. Those septa are in some species strongly thickened, in others remain thin. The conidia are often marked by fine longitudinal and transverse parallel lines of blunt or sharp verrucosities, 0.6 to 2 μ apart and 0.6 to 1.5 μ tall. Sometimes these can be observed only by the use of an immersion lens and may show more clearly in spores that are not quite mature, or only on the basal or apical portion of the spores.

The fungus may appear to the naked eye or hand lens like minute sori of rusts, but under the compound microscope its true nature can be recognized.

After studying type or authentic specimens of all of the eight species recognized as valid by the author, the generic characters may be stated as follows: Parasitic within leaves of Mimosaceae but not producing conspicuously margined leaf spots. Conidiophores hypophyllous, arising from a subepidermal, pale-brown or almost hyaline hypostroma which, without rupturing the epidermis, sends through the stoma balloon-like, almost colorless megalophyses, or which bursts through the stoma and adjacent portions of the epidermis as a small or large, low, more or less flattened sporodochium within which several megalophyses develop. On the separate balloon-shaped megalophyses emerging through individual stomata, or at the tops of the megalophyses making up the upper part of the emerged sporodochium, or from cells of varied size in the absence of distinguishable megalophyses, arise the conidiophores. These are slender or spatulate or spoon-shaped, straight or outwardly and upwardly curved. They are darker in color than the cells of the hypostroma. They may be without transverse septa, but in some species are all transversely septate a short distance from the base. At or near the apex is borne the single conidium. These conidiophores differ from those of *Cercospora* in being unbranched, not branching sympodially and forming a somewhat zig-zag structure as in the latter genus. The conidia differ in their shape from those of *Cercospora* and *Helminthosporium,* rarely averaging more than 2.5 to 5 times as long as broad, and usually being only light brown in color. In shape they may be oblong, nearly equal at each end or more often they are broader near the base and obclavate to obpyriform or lanceolate in outline, sometimes with an obtuse tail. The spores may be straight but often are somewhat curved. They are more often 2–3 times transversely septate, but in one species are regularly 1-septate and in one species 3–4-septate. They are conspicuously roughened or the roughenings may be observed with difficulty if at all.

The genus may be divided into two subgenera distinguished as follows:

Eucamptomeris. Conidiophore-bearing cells (megalophyses) emerging through the stomata singly from the subepidermal hypostroma and bearing the conidiophores apically and laterally. Type species of the genus and subgenus, *Camptomeris calliandrae* Sydow. (See FIGS. 3, 4, 6, 7, 10, 11, 13, 14, 16, 17, 18.)

Exosporioides. Hypostroma bursting through the stoma and sometimes rolling back a considerable amount of the epidermis and cuticle and forming a more or less well marked, mostly flat-topped sporodochium. From enlarged cells (megalophyses) arising from the small basal cells or from branching hyphae or from scattered enlarged cells in the sporodochium arise 2 to 10 conidiophores to each enlarged cell, those nearer the center straighter and more isodiametric, those nearer the margin flattened and bowed outward and upward. Mostly in this subgenus the conidiophores are not so definitely transversely septate near the base as in some species of the subgenus *Eucamptomeris.* Type species of the subgenus, *Camptomeris leucaenae* (Stevens and Dalbey) Sydow. (See FIGS. 22, 23, 26, 29, 30.)

The specific characters in the subgenus *Eucamptomeris* are based chiefly upon the shape and size of the megalophyses, the number and size of the conidiophores and the presence or absence of transverse septa, and the shape and size of the conidia. In the subgenus *Exosporioides* the size and shape of the sporodochium, presence or absence of megalophysis-like cells, shape and size of conidiophores and conidia are important characters.

In the key and in the descriptions of the species the term megalophysis is applied to the large cells arising from the hypostroma or occupying a more or less definite layer in the sporodochium, from whose tops and sides arise the conidiophores. The term sporodochium is used for the cushion-like stroma which is the outer part of the hypostroma which has burst through a considerable area of the epidermis, rolling it and the cuticle back. The term sporodochidium is applied to the cluster of conidiophores and other hyphae growing out of the more-or-less balloon-shaped megalophysis pushed out through a stomatal opening leaving the remainder of the hypostroma beneath the epidermis.

Camptomeris was first named as a distinct genus in 1927. Clements and Shear (1931) did not recognize its validity as a distinct genus but included it under the genus described by Earle in 1900 under the name *Cercosporidium*. The non-validity of this rests upon the type of conidiophores, which in the latter genus are sympodially branched and zigzag while in *Camptomeris* they are unbranched and bear but a single conidium.

The eight species recognized by the writer are distinguished in the accompanying key which is followed by the detailed descriptions of the species and comments on excluded species and species that have been mentioned in connection with some valid species of *Camptomeris*.

The proper placement of the genus *Camptomeris* in the artificial system of classification of the Fungi Imperfecti raises some questions. Because of the pigmentation of the conidiophores and spores and sometimes of the hypostroma the genus must be placed among the pigmented groups (*i.e.* the "Phaeo" groups). The subgenus *Exosporioides,* with its rather definite sporodochium, would fall naturally into the family Tuberculariaceae. Only by a straining of the definition can the species of the subgenus *Eucamptomeris* be considered as belonging to this family, for the sporodochidium differs fundamentally from a typical sporodochium. Yet the two subgenera seem to be rather closely related. The type of conidiophore, with its single terminal conidium, distinguishes *Camptomeris* from *Cercospora* and *Cercosporidium,* in both of which the conidiophores are of the zigzag type due to the sympodial mode of branching, with several conidia, one at each joint. *Stigmina* shows many similarities to *Camptomeris* but the limitation of the latter to parasitism in the single family Mimosaceae, the occurrence of special enlarged cells (megalophyses) from which arise the conidiophores, and the flattened nature of the outer conidiophores of a cluster seem to be distinct enough to warrant this separation. *Helminthosporium* is much more deeply colored in most species and usually the spores have more numerous septa. Also in general the conidiophore may bear a number of conidia. Many species of *Helminthosporium* may be saprophytes. If there is any close connection between *Camptomeris* and *Helminthosporium* it is

among the leaf parasites of the latter genus possessing rather short conidiophores bearing each a single conidium. What the perfect stage of *Camptomeris* is, if it still exists, has not been suggested. *C. floridana*, the only species of which a recent collection is available, did not grow when inoculated upon standard laboratory culture media suitable for saprophytic and many parasitic fungi.

CAMPTOMERIS SYDOW

Ann. Mycol. **25**: 142, *f.* 6. 1927.

Eucamptomeris subgen. nov.

Megalophyses plus minusve vesiculiformes; ex hypostromate singulatim per stomata emergentes.

Conidiophore-bearing megalophyses more or less balloon-shaped, emerging through the hypostroma singly through the stomata.

Exosporioides subgen. nov.

Hypostroma per stoma et circumiectam epidermidem et cuticulam emergens et sporodochium formans.

Hypostroma bursting through a stroma and rolling back a considerable amount of epidermis and cuticle and forming a more or less well marked sporodochium.

KEY TO THE RECOGNIZED SPECIES

A. Conidiophore-bearing megalophyses more or less balloon-shaped, emerging from the hypostroma singly through the stomata..... Subgenus *Eucamptomeris*

a. From the megalophysis arise only the conidiophores but not any external hyphae connected with other megalophyses.

b. Conidiophores regularly transversely septate near the base.

c. Conidiophores 6–19 (av. 11) per megalophysis, spreading, 26–35 μ (av. 30 μ) in length; conidia 25–53 × 8–16 μ (av. 41 × 12 μ), 3-septate, oblong to broadly obclavate, rarely curved. On *Calliandra*, Costa Rica.....*C. calliandrae*

cc. Conidiophores 10–46 (av. 26) per megalophysis, semi-erect, 26–51 μ (av. 39 μ) in length; conidia 30–70 × 8–12 μ (av. 47 × 10 μ), 3–4-septate, obclavate, gradually tapering to a narrow, blunt apex, straight or curved. On *Albizzia*, Ceylon and Africa..... *C. albizziae*

bb. Conidiophores not septate near base or only occasionally so.

d. Conidiophores 16–82 (av. 60) per megalophysis, spreading, 17–35 μ (av. 26 μ) in length; conidia 26–47 × 7–11 μ (av. 37 × 9 μ), 3-

septate, oblong, usually slightly curved. On *Acacia*. Natal, S. Africa.....*C. verruculosa*

dd. Conidiophores 16–38 (av. 25) per megalophysis, erect or semi-erect, 25–38 μ (av. 32 μ) in length; conidia 35–62 × 9–13 μ (av. 49 × 11.5 μ), 1- or 3-septate, oblong to narrowly ovate or obpyriform, slightly curved. On *Acacia*. Jamaica..*C. martynii*

aa. From the top and side of the megalophysis arise the conidiophores, but from the lower part there grow out, above the epidermis, short hyphae which merge with those from near-by megalophyses to form a sporodochium-like structure which is connected with the hypostroma only by the emergent megalophyses. Conidiophores 16–105 (av. 41) per megalophysis, upright and spreading, transversely septate near the base, 32–46 μ (av. 39 μ) in length for the outer conidiophores, the inner ones 20–30 μ long; conidia 34–48 × 7–11.5 μ (av. 42 × 10 μ); 1- 2- and 3-septate, oblong to obclavate, usually somewhat curved. On *Pithecellobium*, Florida.....*C. floridana*

AA. Hypostroma bursting through a stoma and rolling back a considerable amount of epidermis and cuticle and forming a more or less well marked sporodochium.....Subgenus *Exosporioides*

e. Conidia 1-septate, not or scarcely narrowed toward the apex, 29–49 × 9–12 μ (av. 41.2 × 10 μ); sporodochia 115–235 μ (av. 188 μ) in length, with a near-basal palisade of megalophyses 24–30 × 7.5–12 μ. On *Desmanthus*. Texas and West Indies.....*C. desmanthi*

ee. Conidia 3-(rarely 2-) septate, distinctly narrowed toward apex, 30–59 × 8.3–10.6 μ (av. 47 × 9.5 μ); sporodochia 80–160 μ (av. 114 μ) broad, with oblong or top-shaped megalophyses, 29–43 × 8.6–14.3 μ, borne on coarse, tubular branching hyphae. On *Leucaena*. West Indies and Venezuela.....*C. leucaenae*

eee. Conidia 3-(rarely 2-) septate, obclavate to obpyriform, the apical cell narrowed to a straight or curved, blunt beak, 20–34 × 6.2–9.3 μ (av. 27.8 × 7.3 μ); sporodochia 22–54 μ in diameter, consisting of a narrow, compacted mass of upright conidiophores from a base of cells 4–5 μ in diameter. On *Albizzia*, India.....*C. albizziicola*

EUCAMPTOMERIS

CAMPTOMERIS CALLIANDRAE Sydow, Ann. Mycol. **25**: 142. *f. 6.* 1927. FIGS. 1–4.

Megalophyses almost hyaline, 14–23 μ (av. 19 μ) in diameter, measuring the broadest portion of the part that has emerged from the stoma. This emergent portion is 10–26 μ (av. 16 μ) in height while the sub-stomatal portion is 5–13 μ (av. 10 μ) in height. The rather dark conidiophores are 6–19 (av. 11) per megalophysis, in one or two series, spreading outward and then curving upward, with one transverse septum near the base, forming a cell about as long as broad. Rarely more than one septum occurs. The outer,

upwardly curving portion of the conidiophore is flattened somewhat so that the radial diameter is considerably less than the tangential, giving a somewhat spatulate appearance when the cluster of spreading conidiophores (sporodochidium) is viewed from above. Because the conidiophores spread so much the megalophysis looks from above like a central hole in the sporodochidium. Conidiophores 26–35 μ (av. 30 μ) in length by 11–16 μ (av. 13 μ) in tangential diameter. Sporodochidia 48–109 μ (av. 75 μ) across, usually scattered or clustered, but not interconnected by hyphae above the epidermis. Conidia borne singly at the apex of the conidiophores, ovoid to nearly cylindrical, considerably or scarcely narrowed toward the obtuse apex, lighter brown than the conidiophores, mostly not constricted at the septa. Septa 3 in the mature conidia, fewer or lacking in the younger spores. Conidia usually slightly curved at the apical one-fourth or one-third, apparently smooth when fully mature, but when immature somewhat roughened, especially near the base as well as the conidiophore itself. Dimensions of conidia 25–53 × 8–16 μ (av. 41 × 12 μ). Distinct spots are not visible on the upper side of the affected leaflet except slightly yellow-green areas without definite margins.

On leaflets of *Calliandra similis* Sprague and Riley. No. *89* of Sydow, Fungi in itinere costaricensi. Descamparados, Costa Rica. Collected by H. Sydow, December 30, 1924. The specimens studied were from the foregoing exsiccatum, lent by the Mycological Herbarium of the U. S. Department of Agriculture, Beltsville, Maryland, through the courtesy of John A. Stevenson.

CAMPTOMERIS ALBIZZIAE (Petch) Mason, apud C. G. Hansford, Proc. Linn. Soc. London. 155th Session, 1942–1943 (Pt. 1): 61, *f. 1–16*. 1943. FIGS. 8–11.

Helminthosporium albizziae Petch, Ann. Roy. Bot. Gard. Paradeniya **4**: 306. 1909.

Megalophyses 9.6–35.2 × 6.4–28.8 μ (av. 19 × 16 μ) in diameter, measuring the portion above the stoma, and in total height, including the portions within and outside the stoma, about 40 μ (probably somewhat contracted because measured from a stained paraffin section). Conidiophores 10–46 (av. 26) per megalophysis, semierect, 26–51 μ (av. 39 μ) in length and 7–8.5 μ across for the inner, not flattened ones, and 10–17 μ in width, for the outer, flattened conidiophores. Sporodochidia 38–162 × 35–118 μ (av. 83 × 73 μ) across, scattered or crowded, not united. Conidia borne singly at

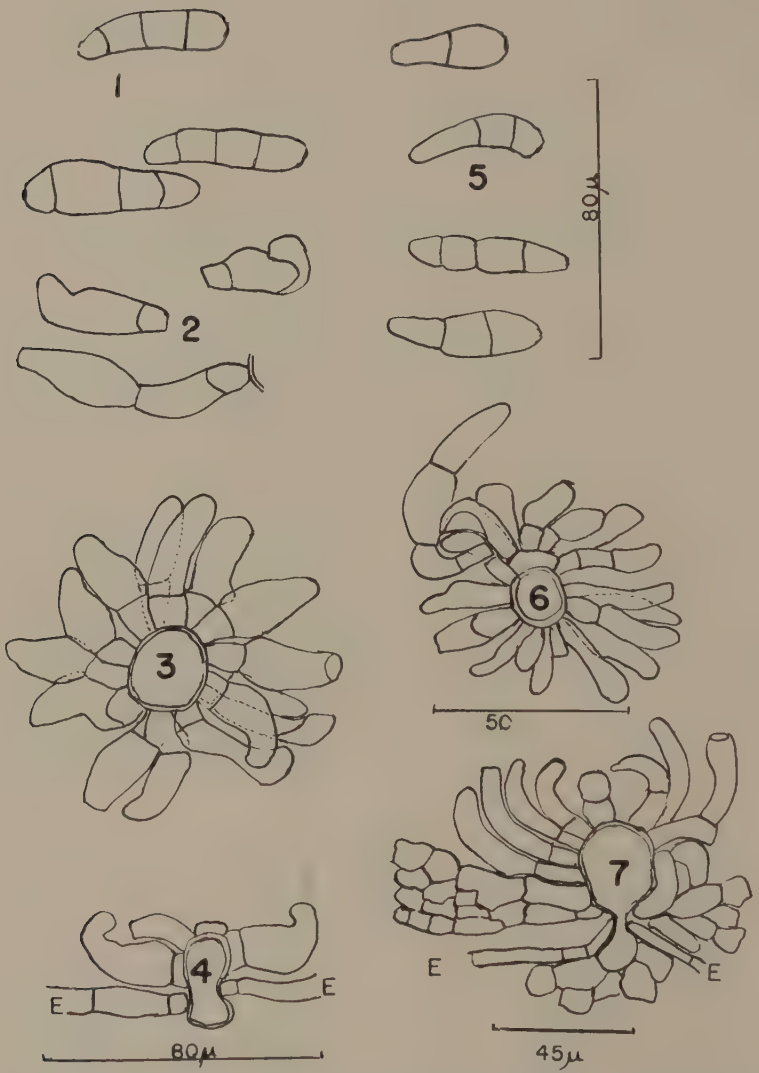
the apex of the conidiophores, obclavate, gradually tapering to a narrow, blunt apex, straight or curved, lighter fuliginous, 30.1–70.4 × 8.3–11.8 μ (av. 47 × 10 μ), 3 to 4 septate (av. 3.5), usually not constricted at the septa, roughened with verrucosities about 1.5 μ apart, less noticeably so on the mature conidia. The dried herbarium specimens (all that were seen) do not show distinctly margined spots on the affected leaves.

Specimens studied: *Helminthosporium albizziae* Petch, type specimen, Petch No. *2589,* on *Albizzia lebbek* (Willd.) Benth., Peradeniya, Ceylon, June 21, 1908 (courtesy Royal Botanical Garden, Kew, England); *Camptomeris albizziae* (Petch) Mason, C. G. Hansford No. *1923,* on *Albizzia grandibracteata* Taubert, Kazi near Kampola, Uganda, Sept. 1936 (courtesy Royal Botanic Garden, Kew, England); C. G. Hansford No. *3458,* on *Albizzia coriaria* Welw., Entebbe Road, Uganda, Sept. 1944 (courtesy of U. S. National Herbarium, through John A. Stevenson); P. Chandler No. *2468,* on *Albizzia* sp., Kampala, Uganda, Sept. 1938 (I.M.I. *7686,* courtesy Commonwealth Mycological Institute); F. C. Deighton No. M. *2591,* on *Albizzia ferruginea* Benth., Njala (Kori), Sierra Leone, Africa, Nov. 28, 1947 (I.M.I. *21151,* courtesy Commonwealth Mycological Institute); S. T. Hughes, on *Albizzia ferruginea* Benth., Gold Coast Colony, Africa (G. C. M. Herb. *1021*), June 13, 1949 (I.M.I. *47542a,* courtesy Commonwealth Mycological Institute).

Camptomeris verruculosa (Sydow) comb. nov. FIGS. 15–18.

Stigmina verruculosa H. & P. Sydow, Ann. Mycol. **10**: 444. 1 *fig.* 1912.

Megalophyses more or less elliptical in cross section, 18–26 × 14–21 μ (av. 22 × 18 μ) in diameter and about 17 μ tall in the portion above the stomatal opening. Conidiophores pale olive-brown, 16–82 (av. about 60) per megalophysis, in about two series, spreading, usually not transversely septate near the base (an occasional conidiophore may be septate), those of the outer series flattened somewhat and slightly curved upward. Conidiophores 17–35 μ (av. 26 μ) in length, in apical view 8.3–11.8 × 6.1–9 μ. Sporodochidia mostly hypophyllous, rarely also epiphyllous, 42–96 μ (av. 71 μ) across, scattered or crowded, but not united. Conidia borne singly at the apex of the conidiophores, oblong, slightly curved,



FIGS. 1–4. *Camptomeris calliandrae* Sydow. 1. Conidia. 2. Conidiophores, one with immature conidium attached. 3. Sporodochidium, from above. 4. Vertical section through megalophysis and attached conidiophores, E. epidermis. FIGS. 5–7. *Camptomeris floridana* Bessey. 5. Conidia. 6. Sporodochidium from below and detached conidiophore with conidium. 7. Vertical section through upper part of hypostroma and megalophysis and attached conidiophores and exostroma, E. epidermis.

26–47 × 7–11 μ (av. 37 × 9 μ), 3-septate (very rarely 2 or 4 septate), not constricted at the septa, pale yellow-brownish. According to Sydow's original description the sporodochidia occupy discolored spots on the leaves.

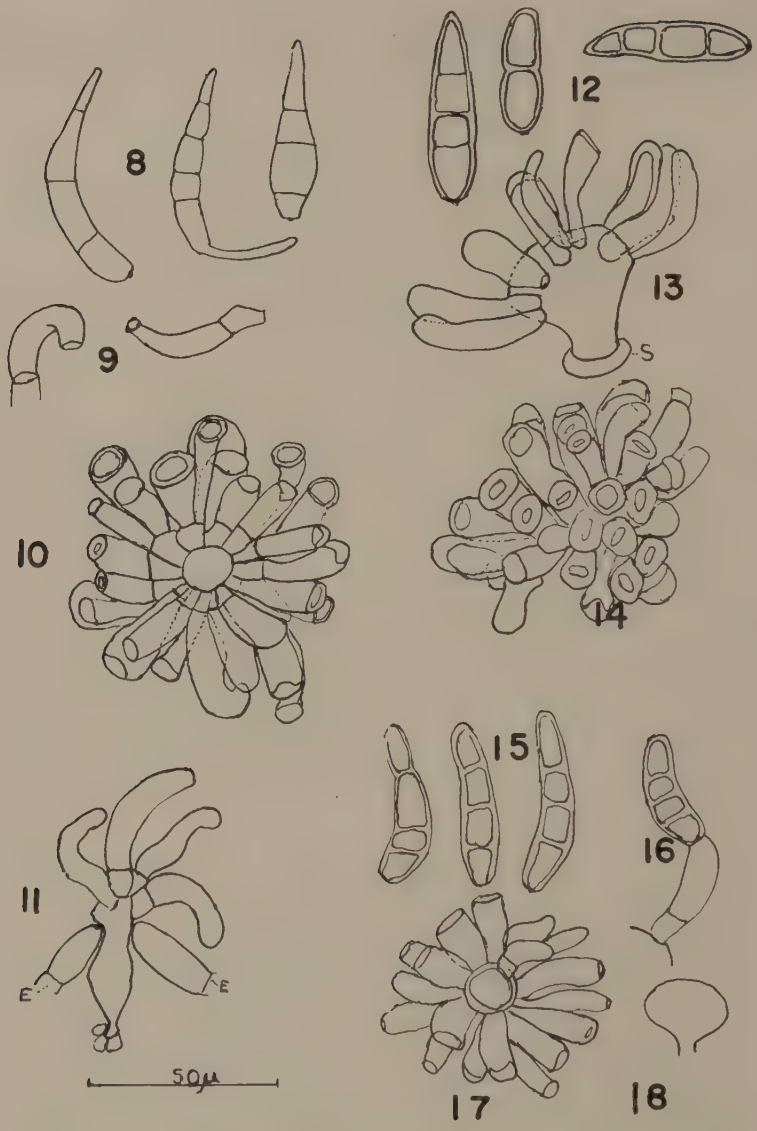
Specimens studied: *Stigmina verruculosa* Sydow (portion of type collection), on *Acacia mollissima* Willd. (= *A. decurrens* Willd.), Harden Heights, Natal, Union of S. Africa, Coll. April 14, 1911 by J. B. Pole Evans, No. *1382* Union of S. Africa Dept. Agr. Myc. Herb. (I.M.I. *38049*, in the *Camptomeris* folder, courtesy Commonwealth Mycological Institute).

Camptomeris martynii sp. nov. FIGS. 12–14.

Megalophyses 12.8–22.4, raro 33 μ (med. 19 μ) diam. in parte suprastomatali et 16–26, raro ad 38 μ (med. 22 μ) alt. supra stoma, fere hyalinae. Conidiophora atro-brunnea, 16–38 (med. 25) in singulis megalophysibus, in pluribus seriebus, erecta seu semi-erecta, exteriora exterius et sursum curvata, et multo applanata, non septata seu raro ad basim septata; 25–38 μ (med. 32 μ) long., exteriora 8–12.8 μ (med. 10 μ) lat., interiora 4.8–9 μ (med. 7 μ) lat. Sporodochidia hypophylla, 55–104 μ (med. 74 μ) lat., sparsa vel aggregata. Conidia singulatim orta ad apices conidiophororum, oblonga seu anguste obpyriformia, e medio ad obtusum apicem angustata, plerumque 3-septata vel frequenter 1-septata, medio septo crassiore quam dua alia, interdum ad septum medium constricta, praecipue conidia immatura exigue verruculosa, pallide brunnea, interdum paululo curvata in dimidio apicali, 35–62 × 9.2–12.8 μ (med. 49 × 11.5 μ).

In foliis vivis *Acaciae villosae* (Sw.) Willd. Long Mountain, Jamaica. Coll. E. B. Martyn, 1949.

Megalophyses 12.8–22.4 μ, rarely 33 μ (av. 19 μ) in greatest diameter of the extrastomatal portion and 16–26, rarely up to 38 μ (av. 22 μ) in height above the stoma, almost hyaline. Conidiophores dark brown, 16–38 (av. 25) per megalophysis, in several series, erect or semi-erect, the outer ones curved outward and upward and flattened considerably, usually not septate but occasionally one transverse septum near the base. Conidiophores 25–38 μ (av. 32 μ) in length, those nearer the margin 8–12.8 μ (av. 10 μ) in breadth, the more centrally placed ones 4.8–9 μ (av. 7 μ) across. Sporodochidia hypophyllous, 55–104 μ (av. 74μ) across, usually scattered, not united even when crowded. Conidia borne singly at the apex of the conidiophore, oblong to narrowly obpyriform, usually narowed from the middle to the blunt apex. Mostly 3-, or often 1-, septate, the middle septum much thicker than the other two, sometimes constricted at the middle septum; very faintly roughened, especially when immature, pale brown, occasionally



FIGS. 8–11. *Camptomeris albizziae* (Petch) Mason. 8. Conidia, one with basal germ tube. 9. Conidiophores. 10. Sporodochidium from above. 11. Megalophysis and attached conidiophores, E. epidermis. FIGS. 12–14. *Camptomeris martynii* Bessey. 12. Conidia. 13. Megalophysis with attached conidiophores, S. stoma. 14. Sporodochidium from above. FIGS. 15–18. *Camptomeris verruculosa* (Syd.) Bessey. 15. Conidia. 16. Conidiophore attached to megalophysis and bearing a conidium. 17. Sporodochidium, from below. 18. Side view of the portion of a megalophysis extending above the stomatal opening.

slightly curved, especially in the apical half; 35–62 × 9.6–12.8 μ (av. 49 × 1.5 μ). No conspicuous spot produced on the affected leaflets so far as visible on the dry herbarium specimen. Named in honor of E. B. Martyn, collector of the type material.

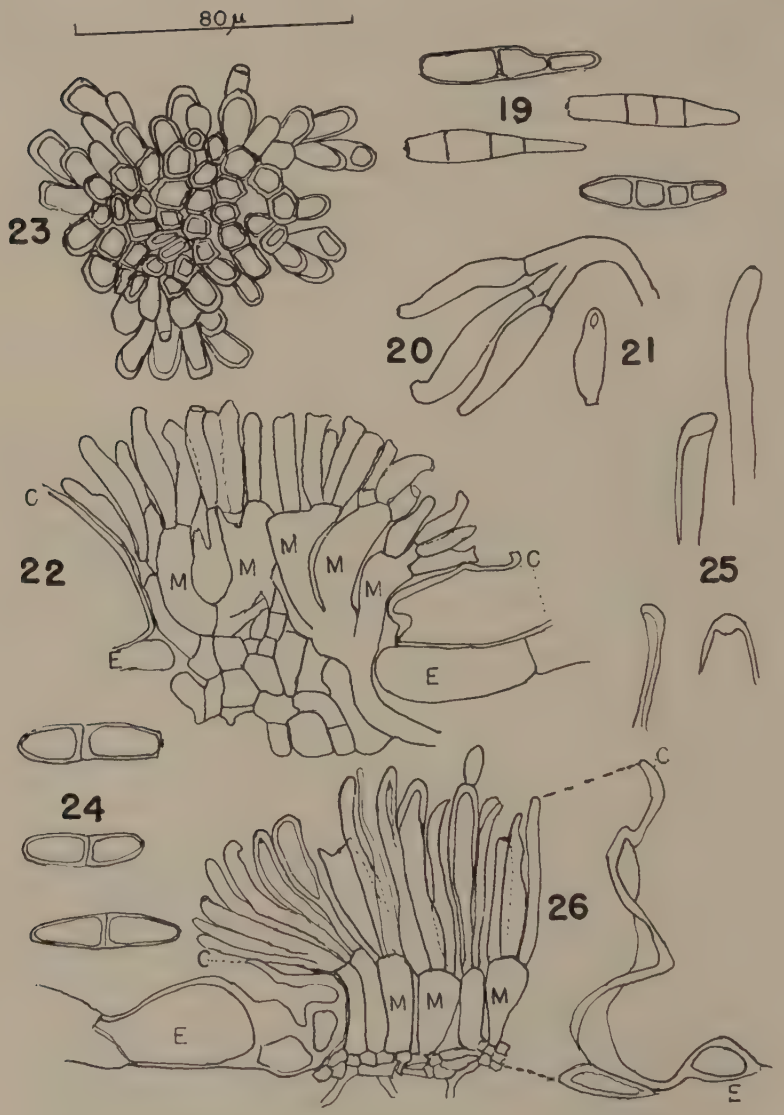
On leaflets of *Acacia villosa* (Sw.) Willd., Long Mountain, Jamaica. Collected by E. B. Martyn, February, 1949. Department of Agriculture, Jamaica, No. P. *18426* (I.M.I. *34795*, courtesy Commonwealth Mycological Institute).

Specimen studied, the foregoing type collection (which is placed in the *Camptomeris* folder, without trivial name).

Camptomeris floridana sp. nov. FIGS. 5–7.

Maculae in foliis hospitis inconspicuae. Sporodochidia hypophylla, raro epiphylla, singulatim vel densiuscule dispersa, subatra, minuta, 86–109 μ diam. Hypostroma pallide ferrugineum, circa 100 μ diam., 25–50 μ crassum. Cellulae hypostromatis pseudoparenchymaticae, 6–14 μ diam., quarum media cellula (megalophysis) sub ostiolo situata, paululo maior. Haec cellula crescit ex ostiolo stomatis et fit vesica 22–42 μ alt. et 18–26 μ lat. Ex huius cellulae superficie oriuntur radiatim multae breves cellulae quarum superiores ferunt conidiophora et ex inferioribus oritur ectostroma. Conidiophora recta seu plus minusve sursum arcuata, cylindracea, 20–46 μ long., 3.5–7.5 μ lat., apicem versus plus minusve attenuata. Conidia singula, acrogena, obclavata vel cylindracea, ad basim rotundata, sursum paululo attenuata, plerumque varie curvata, dilute olivaceo-brunnea, 1–3 septata, ad septa vix constricta, 34–48 × 8–11.5 μ, minute asperulata. Hab. in foliis *Pithecellobii unguis-cati* (L.) Benth. Ft. Myers, Florida, U. S. A. 30. XII. 1950. Leg. Ernst A. Bessey.

Megalophyses 18–26 μ (av. 23 μ) in greatest diameter above the stoma; 22–42 μ (av. 32 μ) in height in the supraepidermal portion, and 16–26 μ in the subepidermal part, the almost hyaline wall of the external portion 1.6–2.5 μ thick. The light brown hypostroma of pseudoparenchymatous tissue is made up of thin walled cells 6–14 μ in diameter and is up to 100 μ or more in width and projects 25–50 μ into the mesophyll, with a few very fine, hyaline hyphae extending to the upper epidermis. The host cells, except where crushed by the stromatic tissue, appear to remain alive although their walls are browned, often clear to the upper epidermis. From the external portion of the megalophysis arise numerous, dark, closely packed cells. Those arising nearer the epidermis multiply and form a mass of stromatic tissue or sporodochium, which remains free from the epidermis. Spreading laterally it joins with similar masses of cells from other megalophyses near by. The cells growing from the upper half of the megalophysis give



FIGS. 19–23. *Camptomeris leucaenae* (Stevens & Dalbey) Sydow. 19. Conidia. 20. Branch of sporodochium bearing three conidiophores. 21. Conidiophore. 22. Vertical section through sporodochium, C. cuticle, E. epidermis, M. Megalophyses bearing conidiophores. 23. Sporodochium from above. FIGS. 24–26. *Camptomeris desmanthi* Ciferri. 24. Conidia. 25. Upper portion of conidiophores. 26. Vertical section through sporodochium, C. cuticle, E. epidermis, M. Megalophyses bearing conidiophores.

rise to the conidiophores, one or rarely two or even three arising from each of these cells. In the cases where only one conidiophore develops the basal cell probably is homologous to the basal cell of the conidiophore as found in other species of *Camptomeris*. The conidiophores from nearer the top of the megalophysis are shorter than those arising from the sides. Inner conidiophores 20–30 μ in length and 3.5–7.5 μ in thickness, sometimes slightly more slender toward the apex, the outer ones 32–46 μ (av. 39 μ) long. They are curved upward and inward. Each bears a single apical conidium. The sporodochidia all arise independently and may be scattered but the ectostromata of those arising closer together join to form a united group of 2 to 3 or even 8 to 10. Rarely a few scattered sporodochidia are produced on the upper side of the leaflet. The width of the individual sporodochidia varies from 86–109 μ (av. 99 μ). From above, the megalophysis is sometimes visible but when the sporodochidia are scraped off by a scalpel and viewed from the under side it is very noticeable as an almost round hole. The conidiophores are lighter in color than their supporting cells but are darker than the conidia. Conidia oblong to obclavate, usually somewhat curved, transversely 1- 2- and 3-septate, the septa thin; minutely verruculose, the verrucosities about 1 μ apart in transverse and longitudinal parallel rows about 1 μ apart. 1-septate conidia 34–42 × 9.6–11.5 μ (av. 37 × 10 μ); 2-septate 42–45 × 7–11.2 μ (av. 43 × 10 μ); 3-septate 42–48 × 8–11.5 μ (av. 46 × 10 μ). These form respectively 40, 25, and 35 percent of the total number of the conidia measured. The living, affected host leaflets show from above no definite spots or sometimes a slight yellowish-green discoloration. From below, the numerous sporodochidia appear black to the naked eye and even when viewed through a hand lens resemble very small rust sori.

On leaflets of *Pithecellobium unguis-cati* (L.) Benth. Coll. Ernst A. Bessey, Ft. Myers, Florida. Dec. 30, 1950. Only specimens studied were from the foregoing type collection.

The type collection has been divided and deposited in the following herbaria: Michigan State College, University of Florida, Mycological Herbarium of the U. S. Department of Agriculture, Commonwealth Mycological Institute, New York Botanical Garden.

EXOSPORIOIDES

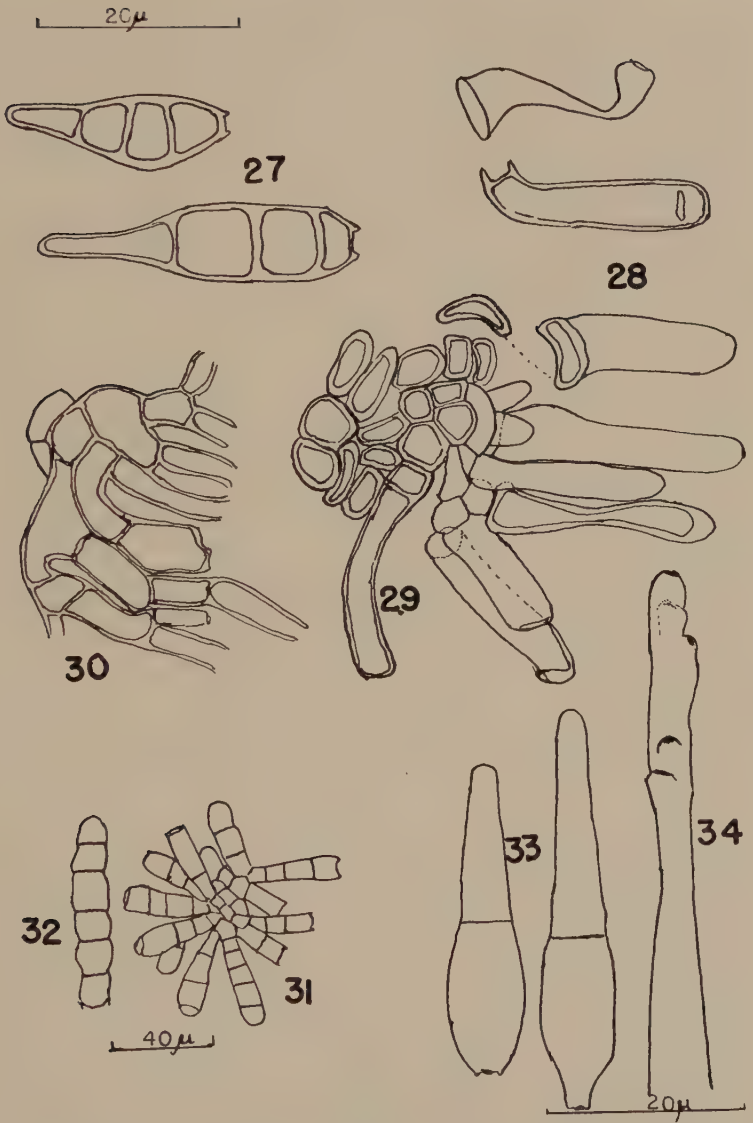
CAMPTOMERIS DESMANTHI Ciferri, Ann. Mycol. **31**: 165. 1933. FIGS. 24–26.

Numerous dark brown sporodochia in hypophyllous patches 2–5 mm. across, on the not conspicuously discolored leaflets. The

sporodochia are elongated in the direction of the main axis of the stomata through which they first burst and measure 115–235 × 100–166 μ (av. 188 × 152 μ). They form rather flat cushions, the upper layer made up almost entirely of the conidiophores. Sections made from paraffin-embedded leaflets show that subepidermally there is a low hypostroma from the upper surface of which arises a layer, more or less palisade-like, of a few up to many enlarged cells (megalophyses) narrowly balloon shaped, measuring 24–30 × 7.5–12 μ. From the upper rounded or flat surface of each megalophysis arise 5 to 6, even up to 10 conidiophores, so that the sporodochium that bursts through the epidermis may be compared to a compound structure of several to many sporodochidia, such as are formed and emerge singly in *Camptomeris calliandrae* and other species in the subgenus *Eucamptomeris*. The conidiophores at the outer edge of each of these small megalophyses curve outward and upward, those nearer the center being less curved and more upright. The conidiophores are dark brown, 45–61 μ in length, those not at the margin being usually 9.6–10.6 × 4.5–7.4 μ (av. 10 × 6 μ) in end view while the marginal ones may be spoon-shaped and in end view up to 13 × 3 μ. They may be somewhat hooked. They bear but one conidium each. They may have a transverse septum near the base forming a cell about 6 μ long and 5 μ broad, but this septum seems to be lacking in many conidiophores (and is not shown in the vertical section of the sporodochium in Fig. 26). The conidia are lighter brown, oblong, usually 1-septate (only one was found with 3 septa). They are usually, but not always, constricted a little at the septum. The septum is 2–4 μ thick, the outer wall of the spore being not so thick. The apical and basal halves of the conidia are quite similar and rounded at the ends. Rarely the distal half is somewhat tapering. They measure 29.4–48.6 × 9–11.8 μ (av. 41 × 10 μ). They are very minutely striate, transversely and longitudinally, with rows of very small verrucosities, visible mostly only under the oil-immersion. The foregoing measurements and the illustrations were all made from the type collection. From W. H. Long No. *6508*, collected at Brownsville, Texas, the conidia measured 34.6–45.8 × 8.6–12 μ (av. 40 × 10 μ).

On *Desmanthus virgatus* (L.) Willd., Valle de Cibao, prov. Santiago, Santiago, Hato del Yaque, Dec. 31, 1930, coll. E. L. Ekman and R. Ciferri.

Camptomeris desmanthi (Ell. & Kell.) Petrak. Petrak, F. Mykologische Notizien XII. No. *762*. Über *Cercospora desmanthi* Ell. & Kell. Ann. Mycol. **32**: 333. 1934, was described from specimens collected by E. L. Ekman in Santo Domingo on



FIGS. 27–30. *Camptomeris albizziicola* (Thirumalachar & Narasimhan) Bessey. 27. Conidia. 28. Conidiophores. 29. Portion of sporodochium from above, with a few attached and detached marginal conidiophores. 30. Vertical section through a portion of a sporodochium. FIGS. 31–32. *Camptomeris tephrosiae* Hansford (after Hansford, 1943). 31. Cluster of conidiophores from above. 32. Conidium. FIGS. 33–34. *Cercospora condensata* var. *desmanthi* Ell. & Kell. 33. Conidia. 34. Conidiophore. From type specimen.

the same host and in the same locality as Ciferri's type collection. The description matches in the main that given a year earlier by Ciferri, but Petrak erroneously identified his fungus with *Cercospora condensata* Ell. & Kell. var. *desmanthi* Ell. & Kell., described in 1885, but later made a distinct species, *Cercospora desmanthi* (Ell. & Kell.) Ell. & Ev. 1887.

Specimens of *Camptomeris desmanthi* Ciferri studied: Ciferri Mycoflora Domingensis *3899*. Hato del Yaque, Santo Domingo. Coll. Ciferri and Ekman, Dec. 13, 1930. This is the same locality as the type collection. (Courtesy of U. S. Department of Agriculture, Mycological Collection.) Other specimens of this same species studied by the writer were the following, labeled *Cercospora desmanthi* Ell. & Kell. W. H. Long Herb. *6163*, on *Desmanthus velutinus* Scheele, San Antonio, Texas, Nov. 8, 1916, and *6508* on *Desmanthus* sp. from Brownsville, Texas, May 27, 1918. On the leaflets of a specimen of *Desmanthus leptolobus* T. & G., Lindheimer, Flora Texas *760* collected at Comanche Springs or New Braunfels, Texas, June 1851, recognizable clusters of this fungus were found. (Specimens made available by courtesy of Dr. G. J. Goodman, of the Herbarium of the University of Oklahoma.) Specimens labeled *Cercospora condensata* Ell. & Kell. var. *desmanthi* Ell. & Kell. and *Cer. desmanthi* (Ell. & Kell.) Ell. & Ev. studied by the writer are discussed in greater detail later in this paper.

CAMPTOMERIS LEUCAENAE (Stevens & Dalbey) Sydow, Ann. Mycol. **28**: 222. 1930. FIGS. 19–23.

Exosporium leucaenae Stevens & Dalbey, Mycologia **11**: 5. Pl. 2, figs. 5–7. 1919.

Sporodochia dark brown, scattered, hypophyllous. Leaflets not conspicuously spotted. Sporodochia not noticeably elongated, 80–160 μ (av. 114 μ), forming rather flat cushions, the upper layer made up almost entirely of the conidiophores. From the basal layer of small cells there arise coarse, branching tubular hyphae with swollen oblong or top-shaped ends (megalophyses) 29–43 × 8.6–14.3 μ. These bear at the flattened top mostly about 3 (sometimes 2 or 4) conidiophores. From the megalophyses nearer the center of the sporodochium the conidiophores are more nearly isodiametric in cross section while from those nearer the margin the conidiophores are more flattened and spatulate or spoon-shaped. In length

they are 32–46 μ. Those nearer the margin measure in end view 9.6–10.2 × 6.4–9 μ. The conidia are 2 or 3 septate, more often the latter, the septa and outer walls of the more mature spores somewhat thickened, 30–59 × 8.3–10.6 μ (av. 47 × 9.5 μ). They are obclavate or narrowly lanceolate in outline, distinctly narrowed in the distal portion, very faintly roughened. The above measurements were made on a portion of the type collection.

Specimens studied: *Exosporium leucaenae* Stevens and Dalbey. Portion of the type collection on *Leucaena glauca* (L.) Benth. Porto Rican Fungi *6792*. Coll. F. L. Stevens. Arecibo, P. R., Jan. 17, 1914 (by courtesy of the Herbarium, University of Illinois); *Comptomeris leucaenae* (Stev. & Dalb.) Syd. on *Leucaena glauca* (L.) Benth., collected by H. Sydow, near Caracas, Venezuela, June 2, 1898 (courtesy Mycological Collection of U. S. Department of Agriculture); *3133* of P. Sintinis, Plantae Portoricenses, Coano, in convalli rivuli Moreno, Puerto Rico, Dec. 18, 1885; coll. R. Ciferri, Santiago, Hato del Yaque, August 1931, I.M.I. Herbarium *3026* ex Petrak, Mycotheca generalis *726* (by courtesy of Commonwealth Mycological Institute).

CAMPTOMERIS ALBIZZIICOLA (Thirumalachar & Narasimhan) comb. nov. FIGS. 27–30.

Helminthosporium albizzicolum Thirumalachar & Narasimhan, Sydowia **4**: 67. 1950.

Sporodochia hypophyllous, numerous, scattered or concentrically arranged, in red-brown spots 2 to 4 or 5 mm. × 1 to 2 mm., 22–54 μ (av. about 36 μ) in diameter, about as long as wide. They apparently start to push through a single stoma but burst this and turn back the surrounding epidermis and cuticle. The top is rather flat and from above only the upper portions of the conidiophores and the freed conidia are visible. At the base is a mass of more or less isodiametric or somewhat elongated dark-walled cells, 4 to 5 μ in diameter, from which arise the conidiophores, packed closely together, those in the center rather straight but towards the margin bowed outward and upward. Conidiophores pale brown, 12–25 μ long, the central ones nearly isodiametric, 3–5 μ across, those near the margin flattened and 6–8 × 2–3 μ in cross section. Some of the marginal ones are hooked at the apex where the scar of the single conidium is sometimes very distinct, often subapical. Some conidiophores appear to have a transverse septum near the base

but possibly the cell cut off by this should be regarded as a cell of the basal sporodochium. Conidia more dilutely brown, obpyriform to broadly obclavate, rounded at the base with a distinct scar of attachment, the distal portion narrowed to a straight or curved obtuse beak; 3- (rarely 2-) septate, the septa and wall of the spore rather thin. Under the oil immersion the whole spore or only its basal and apical portion show a very fine aculeation, the aculeae being 0.6–0.7 μ apart and 0.6–1.5 μ high. Conidia 20–33.6 × 6.2–9.3 μ (av. 28 × 7.3 μ).

Specimens studied: Portion of the type collection of *Helminthosporium albizzicolum* on *Albizzia lebbek*, Tirupati, South India. Coll. M. J. Thirumalachar, Jan. 10, 1946 (courtesy of M. J. Thirumalachar).

SPECIES EXCLUDED

CAMPTOMERIS TEPHROSIAE Hansford, Proc. Linn. Soc. London 155th Session, 1942–1943 (part 1): 61. FIG. 12. 1943. FIGS. 31, 32.

Authentic specimens of this fungus could not be located in the Herbarium of the Commonwealth Mycological Institute nor in the Herbarium of Kew Botanic Garden. On information from E. W. Mason of the former, the Uganda Herbarium is at present not accessible. The following descriptions are taken from the original publication by Hansford:

22. *Camptomeris Tephrosiae* Hansford sp. n. Maculae epiphyllae, flavidae, elongatae, in centro brunneae, hypophyllae, indistinctae, dilute flavo-brunneae. *Sporodochia* plerumque hypophylla, minuta, atra, pulvinata, circa 100 μ diam. *Conidiophora* radianta, plus minusve erecta, olivacco-brunnea, 2–3 septata, haud constricta, simplicia, 30–10 μ, inferne attenuata. *Conidia* clavato-cylindracea, atro-brunnea, levia, 3–5 septata, leniter constricta, basi truncata, apici rotundata, 50–95 × 16–22 μ. Hab. in foliis Tephrosiae candidae, Kampa'a, Hansford 1862.

Causes defoliation of the host; the leaf-spots are lenticular, elongated along the veins of the leaflets. There is little differentiation of the conidiophores and conidia; together each consists of a single row of cells which break off at a septum, the outer wall remaining as a broken ring around the apex of the "conidiophore" and around the base of the "conidium."

By the description and illustration this is certainly not congeneric with *Camptomeris*, but the writer, without opportunity to study au-

thentic specimens, dares not venture to indicate where it belongs. The 2–3-septate conidiophores, the 3–5-septate, clavate-cylindric conidium, which on separation from the conidiophore leaves a broken ring, are characters lacking in typical *Camptomeris*.

CAMPTOMERIS ROBINIAE (Shear) Ciferri, Mycopath. **6**: 25. 1951.
Fusicladium robiniae Shear (1902).

The writer has examined specimens of this fungus collected by J. A. Stevenson and Miss Cash in 1936 at Mountain Lake Biological Station, Virginia, and checked with Shear's type specimen of this fungus, as well as specimens collected by Dr. and Mrs. Shear and distributed in Fungi Columbiani, Century XIII. These showed all the characteristics of *Passalora* and in measurements and other characters of the conidiophores and conidia cannot be distinguished from *P. bacilligera* (Mont. & Fr.) Fr. apud Fres., the type species of the genus, except possibly by the host and the more marked spots on the leaves. This species must, therefore, be excluded from the genus *Camptomeris*.

CAMPTOMERIS ACACIAE (Sydow) Ciferri, Mycopath. **6**: 25. 1951.
Septogloeum acaciae H. Sydow, Ann. Mycol. **12**: 489. 1914.

On *Acacia arabica* Willd. Coimbatore, Madras Pres. India. Coll. W. McRae. Specimens could not be obtained for study. The fact that Sydow states that the conidiophores are lacking ("sporophoris nullis") and that the "acervuli" are subcutaneous and that the spores emerge in light yellow drops would seem to bar this from inclusion in *Camptomeris*.

CERCOSPORA CONDENSATA var. DESMANTHI Ell. & Kell. Journ. Mycol. **1**: 2. 1885.
Cercospora desmanthi (Ell. & Kell.) Ell. & Kell. Jour. Mycol. **3**: 14. 1887.
Cercosporidium desmanthi (Ell. & Kell.) Earle ex Dearness. Mycologia **33**: 365. 1941.

This fungus was described from specimens of *Desmanthus brachylobus* Benth. (more properly *D. illinoensis* (Michx.) MacM.)

collected by W. A. Kellerman at Great Bend, Kansas, August, 1884.

Specimens studied: Kellerman *632*, type specimen, from Great Bend, Kansas, Aug. 1884. Others from Kansas were Kellerman *941*, Manhattan; Kellerman & Swingle, Kansas Fungi *5;* Ellis & Everhart, North American Fungi *1992;* Bartholemew, Fungi of Kansas *1892;* from Missouri, Rabenhorst-Pazschke, Fungi Europaei et extraeuropaei *4192;* from South Dakota, Brenckle Fungi Dakotenses *627;* from Louisiana, Langlois *548*, July 27, 1886. This is the specimen on which the var. *desmanthi* was raised to specific rank; from New Mexico, Earle and Earle, Plants of New Mexico *267;* from Texas, on *Desmanthus acuminatus* Benth., W. H. Long Herbarium *5399* and *6147;* on *D. velutinus* Scheele, W. H. Long Herbarium *6183*. This fungus occurred on a specimen of *Desmanthus illinoensis* in Plants of Oklahoma *5442*, collected in the Arbuckle Mts., Murray Co., Okla.

The compact sporodochia, emerging through the stomatal openings which they soon rupture and greatly enlarge, bear on their flattened surface numerous conidiophores which are compacted together below their free apical portions with measure 35–50 × 3.5–6.2 μ (av. of 5, 43 × 5 μ). These apical portions are not septate, but toward the outer end are somewhat zig-zag, bearing the scars of 4 to 8 spores which have been formed sympodially. In cross section the conidiophores are almost isodiametric. They are not flattened or spoon-shaped, even at the margin of the sporodochium. The conidia are obclavate or narrowly lanceolate in outline, but with a rounded tip. About 67% are 1-septate, the remainder being 3- or 2-septate. They measure 23.1–41.3 × 5.9–8.8 μ (av. 31 × 7.3 μ). The septa and outer wall are not pronouncedly thickened.

Although this fungus possesses the *Cercospora* type of conidiophores, by which it is entirely distinct from *Camptomeris*, the conidia are broadly blunt and only 2.8 to 6.3 times as long as broad at the point of greatest diameter (average ratio of length to breadth 4.3 to 1), whereas in typical *Cercospora* the ratio is 10 to 1 or more. For example, in *Cercospora olivacea* (Berk. & Rav.) Ell., on *Gleditsia triacanthos* L., this ratio as measured by the writer is

8.0–32.5 to 1, averaging 17.1 to 1. The genus *Cercosporidium*, with *C. euphorbiae* (Tracy & Earle) Earle as the type species, was established by Earle (**1900**) to accommodate a fungus with elongated, more or less spreading conidiophores of the sympodial type arising in a sort of sheaf or sporodochium-like structure, but with relatively short conidia. Later a few other species were added and in 1941 Dearness transferred *Cercospora desmanthi* (Ell. & Kell.) Ell. & Ev. to it, crediting the change to Earle. Later, Earle (**1902**) questioned the validity of *Cercosporidium* and transferred its species to the older genus *Passalora*. That this is wrong is made evident by a study of the type species, *P. bacilligera* (Mont. & Fries) Fries ex Fres. The writer has not been able to obtain the type specimen but has studied various exsiccata of this species prepared by such European mycologists as Krieger, Petrak, Tranzschel & Serebrianikow, Kabát & Bubák, Fuckel, Kirulis, Keissler, Sydow, Rabenhorst, Roumeguère, Saccardo, deThümen, Briosi & Cavara, and others, loaned by the generosity of the Mycological Herbarium of the U. S. Department of Agriculture and of the New York Botanical Garden. After a study of these the writer agrees with von Höhnel (**1923**) that *Passalora* is distinguished from *Fusicladium* by the conidiophores of the former breaking out in a bundle through a stomatal opening and after a short distance separating somewhat to form free, mostly unbranched, sparsely, if at all, septate portions 50 to 150 μ long. At the apex of each conidiophore arises a single conidium which is by-passed by the sympodial branching of the conidiophore to bear another conidium, and so on until several, up to 5 or 6 more conidia have been produced, each falling when mature. As a result the apical portion is a little thicker than the main portion of the conidiophore and slightly zigzag and bears the scars of the fallen conidia. The conidia are always uniseptate, rather slender, blunt at the apex, the terminal cell narrower than the somewhat swollen basal cell. The walls and the septum are not much thickened. In *Fusicladium* the conidiophores are formed subcuticularly and are short, unbranched and mostly non-septate, each bearing only a singly apical conidium. Closely related to *Passalora* and distinguished mainly by the number of septa in the conidium is *Cercosporidium*. The type species, *C. euphorbiae* (T.

& E.) Earle (1900), was based on *Scolecotrichum euphorbiae* Tracy & Earle (1896). This was shown by Shear (1902) to be the same as *Fusicladium fasciculatum* Cke. & Ell. (1878) and was transferred to *Scolecotrichum* under the name *S. fasciculatum* (C. & E.) Shear. In 1902 Earle concluded that this was congeneric with *Passalora* and gave it the name of *P. fasciculata* (C. & E.) Earle, transferring *C. euphorbiae* (T. & E.) Earle, and *C. helleri* Earle, to *Passalora*. The writer's study of the type collection of *Scolecotrichum euphorbiae* T. & E. revealed that the conidia are 68% 3-septate, 8% 2-septate and 24% 1-septate. The last were in many cases apparently immature. For this reason he agrees with von Höhnel's conclusion that the genus *Cercosporidium* is valid and that the correct name of the type species is *C. fasciculatum* (C. & E.) Höhn. Because one-third of the conidia are more than 1-septate the writer agrees with Dearness (1941) in placing *Cercospora desmanthi* (E. & K.) Ell. & Ev. in the genus *Cercosporidium* under the name *Cercosporidium desmanthi* (E. & K.) Earle. It has nothing to do with *Camptomeris desmanthi* Cif. and the name *Camptomeris desmanthi* (E. & K.) Petrak is invalid as it is a later name and is based on two distinct fungi. *Cercospora condensata* E. & K. on *Gleditsia triacanthos* L. and *C. olivacea* (B. & Rav.) Ell. on the same host are typical species of *Cercospora* and have nothing to do with *Cercosporidium*.

The writer would urge all mycologists interested in *Camptomeris* and related fungi to make a careful study of all available specimens that have been described as leaf parasites, especially upon Mimosaceae, and perhaps also upon the related Caesalpiniaceae and Fabaceae and ascribed to the genera *Cercospora*, *Cercosporidium*, *Passalora*, *Helminthosporium*, *Stigmina*, and *Exosporium*. It is to be expected that other species of *Camptomeris* may be turned up by such a search.

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P. Rogers, who made loans of specimens from the N. Y. Botanical Garden Herbarium, especially type specimens from the J. B. Ellis herbarium; E. W. Mason, for numerous specimens from the herbarium of the Commonwealth Mycological Institute; the Royal Botanic Garden at Kew, for specimens from the Herbarium; Professor Marie B. Morrow, for specimens from the Herbarium of the University of Texas; Dr. Leland Shanor, for type material of *Exosporium leucaenae* from the Herbarium of the University of Illinois; Dr. M. J. Thirumalachar, for a portion of the type collection of *Helminthosporium albizzicolum;* Dr. C. J. Alexopoulos and Charles L. Gilly, my colleagues in Michigan State College, for advice in matters nomenclatorial, and to others who have assisted me by making slides, etc. Thanks are also due to Dr. G. J. Goodman of the Department of Botany of the University of Oklahoma, who permitted the writer to study the Mimosaceae in the herbarium in the search for species of *Camptomeris* and *Cercosporidium.*

Postscript. While awaiting the printing of the manuscript of this paper there appeared the following publication in which the genus *Camptomeris* was treated: Hughes, S. J. Studies on micro-fungi. XIV. Stigmella, Stigmina, Camptomeris, Polythrincium, and Fusicladiella. Commonwealth Mycol. Inst. Mycol. Papers, No. 49: 1–25. December, 1952. Pages 14–19 and figures 9–13 are devoted to this genus. Hughes recognizes as valid five species: *C. calliandrae, C. leucaenae, C. albizziae, C. desmanthi* and *C. sp.* He includes in *C. albizziae* what is described in this paper as *C. verruculosa* and as *C. sp.* what the writer names *C. martynii.* Hughes did not have available for study material of what the writer names *C. floridana* and *C. albizziicola* and so these are not mentioned. The writer considers that Hughes is in error in including *C. verruculosa* under *C. albizziae.* In his illustrations Hughes shows conidiophores with numerous scars of conidial attachment. Only in one case was the writer able to detect these supplementary conidial sears in the material studied by him. Apparently only one conidium is normally produced on each conidiophore.

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STUDIES IN THE GENUS PLEOSPORA. V [1]

LEWIS E. WEHMEYER

(WITH 18 FIGURES)

In previous papers the writer has discussed the species of *Pleospora* with 3-septate spores (5) and the origin of the vulgaris and leptospheroid series from this group (6). It is the concern of this and a following paper to trace the relationships of a rather heterogeneous group of species arising from the 5-septate level of the leptospheroid and vulgaris series.

It was previously pointed out (5) that *P. vagans* with 5-septate spores has a spore form similar to that of *P. pellita* with 3-septate spores and can be derived from that species by the insertion of a secondary septum in each of the end cells.

Beyond this 5-septate level, the insertion of secondary and tertiary transverse walls in spores of the leptospheroid type has proceeded in an irregular fashion. In spores of this series with five or more septa it is common to find a secondary septation of the vulgaris type in the two central cells. Likewise spores of the vulgaris series, with five septa, may have secondary transverse septa of the leptospheroid type inserted in their end cells. These two types of septation result in a convergence of these two series and, unless the sequence of septation is known, leaves the derivation of such spores in doubt. There are certain characters, however, which aid in placing such spore types.

In spores with a primary leptospheroid septation there is a distinct tendency for an asymmetrical insertion of transverse septa, which is accompanied by an asymmetry of form. This is usually the result of the insertion of secondary or tertiary septa in the lower end of the spore without a corresponding insertion in the upper portion of the spore. For this reason, in the following discussion, the species having spores showing both the vulgaris and lepto-

[1] Papers from the Department of Botany of the University of Michigan, No. 944.

spheroid type of septation are placed in the leptospheroid series if they are asymmetrically septate, and in the vulgaris series if they are symmetrically septate. This, again, is a more or less arbitrary arrangement adopted for clarity of presentation.

In both these series there is a good deal of variation in spore form and this can be used to advantage in judging relationships, for several groups of species stand out in this respect.

The present paper will be restricted to a discussion of the species with leptospheroid or asymmetric septation of the spores.

Inasmuch as nearly all spores above the 5-septate level show some central cells with a vulgaris septation, there is only one group of two species, outside of *P. vagans,* which shows an exclusive leptospheroid and at the same time a symmetrical septation. Both *P. Galii* and *P. Galiorum* (FIGS. 1, 2), of this group, have broad-fusoid, dark red-brown, inequilateral, nonconstricted spores, similar in form to those of the vulgaris series, but having the transverse septa all laid down first and the vertical septa inserted irregularly, and often lacking from some of the cells. The two species differ only in the number of septa and size of the spores. Young spores of *P. Galiorum,* which are normally 7-septate, may have only three or five septa and be about the same size as those of *P. Galii.* Occasionally spores of *P. Galii* are found with only four septa and they then resemble *P. intermedia* in form.

Collections, referred to by number in the text, are listed at the end of the paper.

Pleospora Galii (Romell in Herb.) sp. nov. FIG. 1

Perithecia 300-350 μ diametro, dispersa, depresse globosa, ultime collapsa, erumpentia; basi sparse tomentoso; pariete 10-15 μ crasso, brunneo, parenchymatoso.

Asci numerosi, clavati, 78–90 μ longi, 8.5–11 μ crassi, crasse membranacei, basi unguiculiformes.

Sporae biseriatae, 16–17.5 μ longae, 6 μ crassae, fusiformes vel clavati-ellipsoidales, 5-septatae, brunneae vel atro-brunneae, paululo inaequilaterales vel curvatae, saepe sursum rotundatae, deorsum angustatae, ad septa haud constrictae; cellulis centralis cum septis verticalibus praedita.

Specimen typicum (n. *169*) vide infra enumeratione locorum. In caulibus *Galii veri,* par Huddinge: Huddinge.

Perithecia 300–350 μ in diameter, scattered, flattened-spheric, finally somewhat collapsed, erumpent, slightly tomentose about the base; wall 10–15 μ thick, of small-celled, brown parenchyma.

Asci numerous, clavate, wall slightly thickened, base somewhat tapered and claw-like, 78–90 × 8.5–11 μ.

Spores biseriate, fusoid- or clavate-ellipsoid, 5-septate, dark yellow to red-brown, usually somewhat inequilateral or curved, commonly rounded at one end and more tapered toward the other, without constrictions, vertical walls usually found in one, or several, central cells, 16–17.5 × 6 μ.

Collections: *160* (type), on *Galium.*

These spores and those of the following species might easily be mistaken for members of the vulgaris series. They are distinctive in their inequilateral and non-constricted form. The septation, however, seems to be of the leptosphaeroid type, for the secondary septa are inserted in the end cells, and many spores can be seen with five transverse but no vertical septa.

Pleospora Galiorum sp. nov. FIG. 2

Perithecia 250–300 μ diametro, 150–200 μ alta, dispersa, in cortice vel ligno immersa, ut maculae pustulatae, atrae erumpentia; pariete 10–150 μ crasso.

Asci numerosi, clavati, 65–76 μ longi, 14–15 μ crassi, crasse membranacei, inter pseudoparaphyses immersi.

Sporae biseriatae, fusiformi-ellipsoidales, 17–21.5 μ longae, 7–8.5 μ crassae, 7-septatae, saepe sursum rotundatae, deorsum angustatae, ad septa non constrictae, septis verticalibus in cellulis quibusquibus vel omnibus, rarius in cellulis terminalibus.

Specimen typicum (n. *334*) vide infra in enumeratione locorum. In caulibus *Galii Molluginis,* ex Suecia.

Perithecia 250–300 × 150–200 μ, appearing on the surface as scattered, hemispheric, swollen, blackened spots beneath which they are immersed within the wood fibers or the bark; walls thick (up to 150 μ), black, composed of a mixture of fungous tissue and blackened host tissue.

Asci numerous, clavate, wall not much thickened, 65–76 × 14–15 μ, imbedded in an abundant interthecial tissue.

Spores biseriate, fusoid-ellipsoid, mostly 7-septate, dark yellow-brown, straight, or mostly somewhat inequilateral or curved, often rounded at one end and tapered toward the other, without constrictions or very slightly constricted at the central septum, vertical septa in one or all of the central cells, occasionally in the end cells, 17–21.5 × 7–8.5 μ.

Collection: *334* (type), on *Galium,* from Sweden.

In spore form this collection is identical with that of *P. Galii,* but an additional septum has been inserted in each end cell. The perithecia however, are somewhat flattened, strongly erumpent and with a perithecial wall that is almost colorless below, in which respect it approaches a *Teichospora* or even a *Hysterographium.*

There is a second collection (*776*), in the Riksmuseum, on *Barbaraea,* which has spores (19.5–23 × 7–9 μ) almost identical to those of *P. Galiorum,* but with more elongate (800–1000 × 300–400 μ) hysteriaceous fruiting bodies, again with a faint indefinite lower well. More collections are needed to determine whether these two collections represent the same species and if so whether it should be considered a *Hysterographium* or a *Pleospora.*

The two following species are unique in the cylindric form of the spore, with one cell enlarged.

PLEOSPORA OBLONGATA Niessl, Not. Pyr. (Verhandl. nat. Ver. Brünn **14**: 177). 1876. FIG. 5

Pleospora oblongispora Rehm, Ann. Myc. **5**: 542. 1907.

Perithecia 150–250 μ in diameter, globose or somewhat flattened, thickly scattered, remaining immersed, erumpent as a short stout ostiolar neck; walls 10–20 μ thick, parenchymatic, smooth.

Asci clavate to cylindric-clavate, wall somewhat thickened, base claw-like, 65–90 × 9–13 μ.

Spores biseriate to uniseriate, oblong, cylindric, yellow-brown to dark yellow-brown, 4–5-septate, ends broadly rounded, not constricted or very slightly constricted at the septa, 5th septum, when

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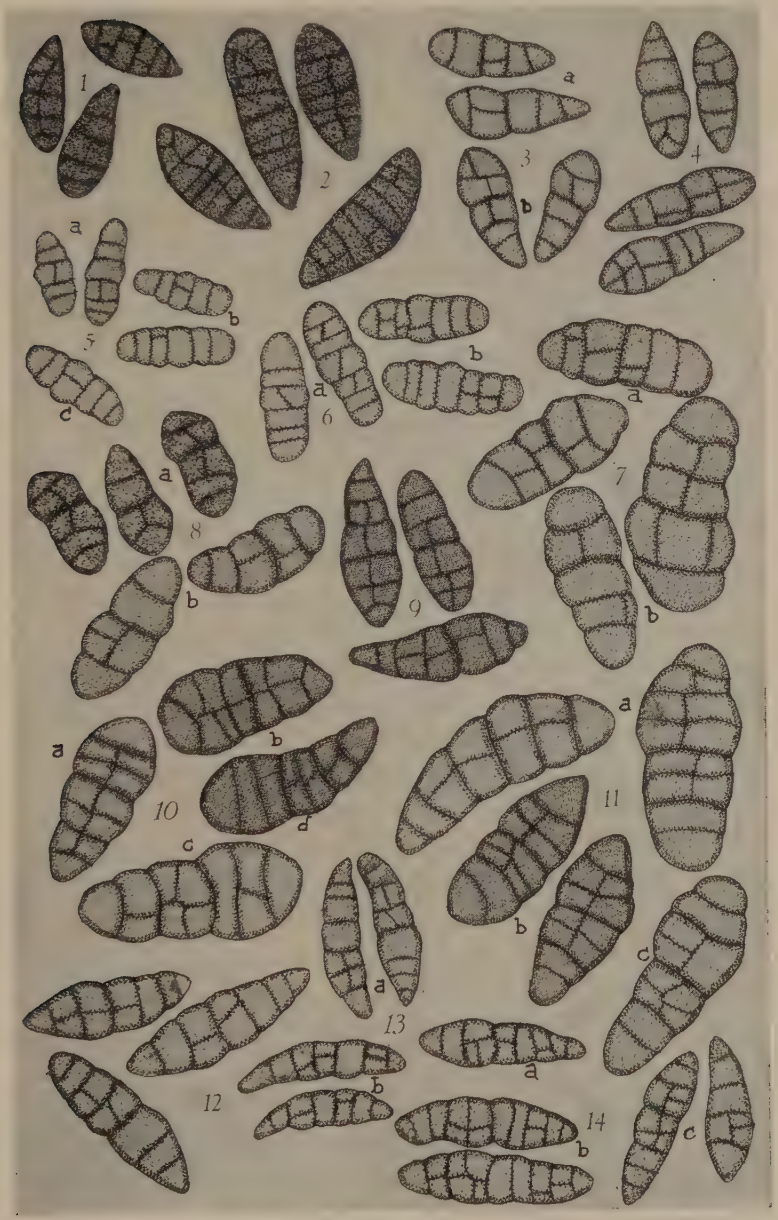
FIGS. 1–14. Ascospores of *Pleospora Galii* sp. nov., from type collection (*160*). 2. *Pleospora Galiorum* sp. nov., from the type collection (*334*). 3. *Pleospora kansensis* E. & E., from collection No. *42* (a) and No. *426* (b). 4. *Pleospora clavispora* sp. nov., from the type collection, No. *337*. 5. *Pleospora oblongata* Niessl, from collections No. *36* (a), *37* (b) and *38* (c). 6. *Pleospora cylindrospora* sp. nov., from the type collection, No. *39* (b) and collection No. *40* (a). 7. *Pleospora Clementsii* sp. nov., from the type collection, No. *43* (b) and from collection No. *82* (a). 8. *Pleospora dichromatricha* (Speg.) Wehm. var. *dichromatricha,* from the type collection, No. *427* (b) and var. *nigriseda,* from collection No. *41* (a). 9. *Pleospora intermedia* Speg., from the type collection, No. *44*. 10. *Pleospora orbicularis* Auersw., from collections No. *46* (a), No. *45* (b), No. *498* (c) and No. *48* (d). 11. *Pleospora trevoicola* Speg., from the type collection, No. *490* (b) and collections No. *347a* (a) and *501* (c). 12. *Pleospora Ilicis* sp. nov., from the type collection, No. *33*. 13. *Pleospora coronata* Niessl, from collections No. *120* (a) and No. *421* (b). 14. *Pleospora dura* Niessl, from the co-type collection, No. *161* (a) and collections No. *121* (b) and No. *122* (c).



FIGS. 1–14.

present, inserted in the upper half of the spore, 3rd or 4th cell somewhat enlarged, vertical walls in one or several of the central, or sometimes the end cells, 12.5–18 × 4.5–6 μ.

Collections: *36, 37, 38*, on *Cirsium, Galium* and *Larix*, from central Europe.

The spores of this species are easily distinguished by their oblong form; they may be either 4- or 5-septate, with the insertion of the extra (5th) septum in the upper half of the spore to give a symmetric septation. If this is the interpretation, the swollen cell occurs below the primary septum and is centrally located in an asymmetrically (**4**) septate spore, which is unusual (see discussion of *L. obtusispora* under next species).

Exsiccati of this species show mixtures of this and the next species as well as other Pleosporas. Niessl, however, gives the spores of his *P. oblongata* as "5 (rarius 4)" septate, and Berlese's figures (2: *Pl. 11, fig. 1*), taken from Niessl's material, are typical of the species here described with 4–5 septa. Berlese's placement of *Pyrenophora Sedi* Roum. & Brun. as a synonym of *P. oblongata* is incorrect, however. The type of *P. Sedi* (*57*) shows a *Pleospora* near *P. vulgaris*, and the description states that it is near *P. chrysospora* (Niessl) Sacc., which would indicate a similar spore form.

The type collection of *P. oblongispora* Rehm (*38*) (FIG. 5c), on *Larix*, shows the same 4- to 5-septate spores. Rehm describes them as 5-septate and 15–18 × 6–7 μ. The perithecia occur on the leaf bases of small young twigs.

Pleospora cylindrospora sp. nov. FIG. 6

Perithecia 200–250 μ diametro, globosa vel modice depressa, dispersa, immersa; ostiolo papillaeformi, erumpente; pariete parenchymatoso, 10–20 μ crasso.

Asci clavati vel longe clavati, 85–110 μ longi, 12.5–14 μ crassi, modice crasse membranosi, basi unguiculiformes.

Sporae biseriatae vel uniseriatae, oblonge cylindricae, 18–21 μ longae, 5.5–7 μ crassae, lutei-brunneae, 5- vel plerumque 6-septatae, utrinque late rotundatae, ad septa non constrictae, cellula quarta inflata, septis verticalibus in cellulis centralibus, interdum in cellulis terminalibus.

Specimen typicum (n. *39*) vide infra in enumeratione locorum. In caulibus herbacearum.

Perithecia 200–250 μ in diameter, globose or slightly flattened, thickly scattered, long immersed, erumpent as a small papillate ostiole; walls parenchymatic, 10–20 μ thick.

Asci clavate to clavate-cylindric, walls somewhat thickened, base claw-like, 85–110 × 12.5–14 μ.

Spores biseriate to uniseriate, oblong, cylindric, yellow-brown to dark yellow-brown, 5- or mostly 6-septate, ends broadly rounded, not constricted at the septa, fourth cell somewhat swollen or enlarged, vertical walls occurring in one or several of the central or, sometimes, the end cells, 18–21 × 5.5–7 μ.

Collections: *39* (type) *40*, on herbaceous stems, Central Europe.

This species is obviously derived from the last, by the insertion of an extra (6th) septum in the lower half of the spore. The spores are slightly larger than in *P. oblongata*, but the less common 5-septate ones have the same form, with the swollen cell below the central septum. The insertion of the sixth septum again places this swollen cell in a symmetric position, with two septa on either side of it. No. *39* is selected as the type because it is the earlier collection. On No. *40*, there is an almost illegible notation "var. . . . septata," so that Rehm also noted the difference in septation.

It is possible, of course, that further collections will show intermediates between this and *P. oblongata*, but all collections so far seen have been quite distinct. *Leptosphaeria obtusispora* Speg., on *Yucca gloriosa*, from Argentina, is interesting in this connection. Berlese (2: 9) states that the spores of this species recall those of *P. oblongata*. The type collection (La Plata Mus. No. *2393*) shows spores of this same general form, 16–21.5 × 4–5 μ. They are rather narrow, but cover the range in length of both the species just described. They are mostly 5-, rarely 4-septate, but show no vertical septa in any of the spores. The swollen cell in this species, however, lies above the central septum and is the third cell in a 5-septate spore.

The remainder of the leptospheroid series show an asymmetric septation and ascospores which are fusoid, ellipsoid or clavate. In the first group, with 4-septate ascospores, *Pleospora kansensis* (FIG. 3) has spores which are easily derived from the 3-septate spores of *P. pellita* by the insertion of an extra septum in the lower end of the spore. The collection (*23*) of *P. parvula*, pre-

viously discussed (5) under *P. vagans*, has this same type of spore, except that in *P. parvula* most of the spores also have an extra septum in the upper half of the spore, making them 5-septate.

P. clavispora (FIG. 4) represents a woody form of *P. kansensis* (FIG. 3). *P. dichromatricha* (FIG. 8), and *P. Clementsii* (FIG. 7), which differ only in the size of their spores, both differ from *P. kansensis* in the darker brown spores with more broadly rounded ends.

The next group which has, typically, six septa in its spores, is apparently derived from a spore type with five septa, by the insertion of an additional septum in the lower end of the spore. This tendency to form additional septa in the lower end appears to be, again, the result of the space relationships in spores which tend to be clavate, with a broad rounded upper portion which is shorter.

TABLE I

Coll. No.	Host	Spores	Asci	Perithecia
		Pleospora oblongata		
36	Galium	12.5–15 × 4–5.5	62–70 × 9–11	200–250
37	Cirsium	14–16 × 5.5–6	70–90 × 10–12	150–250
38	Larix	14–18 × 5–6	62–85 × 11–13	150–200
		Pleospora cylindrophora		
39	Stems	18–21.5 × 6–7	85–100 × 12.5–14	200–250
40	Stems	19–21.5 × 5.5–6	90–110 × 10–13	200–250
		Pleospora kansensis		
426	Azorella	16–19.5 × 7	53–62 × 11–14	200
42	Melilotus	18–21.5 × 7–8.5	55–65 × 14–18	150–250
		Pleospora dichromatricha		
41	Juncus	16–21.5 × 7–9	60–78 × 19–21.5	150–175
427	Euphorbia	19.5–21.5 (28) × 8.5–9	70–85 × 10–11	180–250
		Pleospora Clementsii		
82	Mentha	23–26 × 9–12	75–90 × 21–22	200–300
43	Touteria	26–32 × 12–14	85–90 × 23–25	200–300

TABLE I—*Continued*

Coll. No.	Host	Spores	Asci	Perithecia
		Pleospora orbicularis		
45	Allium	21.5–26.5 × 12–13	85–100 × 21–26	150–300
46	Berberis	23–28 × 10–11	85–90 × 21–26	200–250
47	Berberis	26–30 × 10–12.5	75–85 × 26–28	300–400
48	Berberis	26–32 × 10–12.5	85–90 × 16–18	200–300
49	Berberis	26–33.5 × 11–13	85–90 × 23–27	300–350
503	Colutea	30–35 × 12–14	90–125 × 25–27	150–250
498	Halimodendron	30–36 × 14–16	105–125 × 35	300–400
		Pleospora coronata		
120	Salsola	19.5–25 × 4.5–6	70–78 × 8.5–9.5	200–300
421	Carduus	19.5–26.5 × 5.5–6.5	62–78 × 12.5	200–400
		Pleospora dura		
121	Melilotus	20–25 × 7	74–90 × 12.5	250–350
122	Linaria	23–25 × 7–8	75–90 × 12.5–14	250–300
161	Melilotus	23–30 × 7–9	70–90 × 14–16	300–350
		Pleospora abscondita		
350	Phragmites	28–32 × 7–9	140–160 × 19–23	500x250
162	Aconitum	32–41 × 7.5–10	80–90 × 14	400–500
543a	Silene	37–48 × 9–11		
		Pleospora trevoicola		
490	Trevoa	19.5–25 × 9.5–11	70–78 × 17–19	200–300
501	Genista	25.5–28.5 × 9–10.5	88–97 × 21–23	200–250
347a	Arenaria	33–37 × 11.5–13	125–140 × 26–30	100–150

and a tapered lower portion which is longer and so allows additional septa to be formed therein, below the primary septum. In *P. intermedia* (FIG. 9) the spores are rather narrow and fusoid-clavate, whereas in *P. orbicularis* (FIG. 10) the spores are broader and more definitely clavate. It should be noted that this same tendency in the herbarum series results in asymmetrically septate spores, beyond the 7-septate level (6). In the collections placed in *P. trevoicola* (FIG. 11), there is a greater tendency for an additional septum to occur in the upper portion of the spore, also resulting in 7-septate spores. As a result, the variations in spore form

and septation of this species bridge the gap between a number of species in the leptospheroid, vulgaris and herbarum series, with 5- to 7-septate spores.

PLEOSPORA KANSENSIS E. & E. Journ. Myc. 8: 17. 1902. FIG. 3

Pyrenophora antarctica Speg. Fung. Pat. (Bol. Acad. Cien. d. Cordoba 11: 51). 1887.

Pleospora antarctica (Speg.) Sacc. Syll. Fung. 9: 877. 1891.

Perithecia 150–200 μ in diameter, rather widely scattered, globose to depressed or collapsing; with a penicillate fascicle of hyphae 35–50 μ in length about the ostiole in some cases, wall membranous, 10 μ thick, parenchymatic.

Asci rather stout clavate, apical wall somewhat thickened, base claw-like, 53–65 × 11–18 μ.

Spores biseriate to overlapping uniseriate, fusoid-ellipsoid to clavate-fusoid, 3- to 4- (rarely 5-) septate, yellow-brown to dark yellow-brown, often asymmetric, broader and rounded above, tapered below, mostly inequilateral, constricted at the central septum but only slightly so at the other septa, usually with one septum above and two below the central primary septum, vertical septa in one or more of the central cells, rarely in the end cell, rarely with a vulgaris-type septation in the upper central cell, 16–21 × 7–8.5 μ.

Collections: *42* (type), *426:* On *Melilotus* and *Alsorella*, from Kansas and Patagonia.

The type of *P. kansensis* (*42*) (FIG. 3a) includes good material with perithecia immersed in stems of *Melilotus*. The type of *P. antarctica* (*426*) (FIG. 3B) contains two fungi on leaves of *Alsorella*. One of these has small perithecia with dark brown stiff setae, but the spores in these perithecia are all dark red-brown, almost opaque, collapsed, one- or two-celled and obviously abnormal. A few perithecia with penicillate ostioles were found which contained yellow-brown spores very similar to those described above for *P. kansensis* and agreeing with the drawings on the packet and Spegazzini's description of *Pyrenophora antarctica*. Spegazzini described both a *Pleospora* and a *Pyrenophora antarctica* but Saccardo listed them both under *Pleospora* in his Sylloge (4: 877 & 888). The specific epithet of *antarctica* must be retained for Spegazzini's species of *Pleospora,* however, and the epithet *kansensis* is, therefore, used for this species.

Pleospora clavispora sp. nov. FIG. 4

Perithecia 400–500 μ diametro, 250–300 μ alta, globosa, dense dispersa, in cortice immersa, erumpentia; ostiolo papilliformi; pariete 20–50 μ crasso, parenchymatoso.

Asci longe cylindrici-clavati, 85–110 μ longi, 12.5 μ crassi, crasse membranacei, basi unguiculiformes.

Sporae imbricati-uniseriatae, fusiformi-ellipsoidales vel clavatae, 16–21.5 μ longae, 7–8.5 μ crassae, lutei-brunneae, utrinque angustatae vel sursum rotundatae et deorsum angustatae, plerumque cum 1 septo super et 2 septis sub septo primari centrali; septis verticalibus in cellulis centralibus vel interdum in cellulis terminalibus.

Specimen typicum (n. *337*) vide infra in enumeratione locorum. In caulibus *Sorbi Aucupariae,* ex Suecica.

Perithecia rather thickly scattered, 400–500 × 250 × 300 μ, flattened spheric, imbedded in the bark fibers, erumpent as small papillate ostioles, walls 20–30 or 50 μ (at the sides) thick, composed of small-celled parenchyma.

Asci long, cylindric-clavate, walls somewhat thickened, base claw-like, 85–110 × 12.5 μ.

Spores overlapping uniseriate, fusoid-ellipsoid to clavate, mostly 4-septate, rarely 3- or 5-septate, light yellow-brown, both ends tapered or, more commonly, the upper end rounded and the lower end tapered, usually with one septum above and two below the central primary septum, vertical septa in the central cells, or sometimes in the end cells, 16–21.5 × 7–8.5 μ.

Collection: *337* (type), on *Sorbus Aucuparia,* from Sweden.

This species is based upon a single undetermined collection in the Riksmuseum Herbarium, at Stockholm. One packet contains perithecia on the inner surface of bark of *Sorbus* and a second packet, from the same locality and the same date, shows perithecia in the bark fibers remaining upon the surface of a reddish discoloration on a decorticated twig. They are both the same fungus and it differs from *P. kansensis* in the larger perithecia, the longer, more cylindric asci and the spores, which are more commonly 3- or 5-septate and have vertical septa in the end cells.

Pleospora dichromatricha (Speg.) comb. nov. FIG. 8

Perithecia 150–250 μ in diameter, either widely or thickly scattered, spheric or flattened, sometimes collapsing, immersed, slightly pustulate, ostiole punctate, wall thin, either smooth or covered over the entire surface with stiff dark brown hairs.

Asci cylindric-clavate to stout clavate, with thickened wall and claw-like base, 60–100 × 10–20 μ.

Spores biseriate, ellipsoid, red-brown, 3- to 5-, mostly 4-septate, with one septum above and two below the central primary septum, mostly straight and somewhat asymmetrical, ends broadly rounded, tapered below the middle, 16–21.5 × 7–9 μ.

Collections: *427* (type) and *41*, on *Euphorbia* and *Juncus*, from the Canary Islands and the Caucasus.

These two collections may prove to be distinct species, but until more and better material can be had for study they are placed here as separate varieties under one species, because of the similarity of their spores.

var. **dichromatricha** stat. nov. FIG. 8b

Pyrenophora dichromatricha Speg. Anal. Mus. Nac. Buenos Aires **26**: 126. 1914.

Perithecia 180–250 μ in diameter, spheric or flattened to somewhat collapsed, covered with a sparse to more abundant tomentum of stiff brown hairs. Asci rather cylindric-clavate, 75–100 × 10–20 μ. Spores 19.5–21.5 μ.

Collection: *427*, on *Euphorbia*, from the Canary Islands.

The type of *Pyrenophora dichromatricha* Speg. has rather agglutinated spores. The spores seen from asci or perithecia measured 19.5–21.5 × 8.5–9 μ. There were also spores scattered about upon the surface of these stems which appeared swollen, were larger (23–28 × 10–12.5 μ), lighter yellow-brown, and commonly showed a fifth septum. These appeared to be exuded spores which were about to germinate. Judging from Spegazzini's description and drawings upon the type packet, these exuded spores were the ones used as a basis for his species.

var. **nigriseda** (Rehm) comb. nov. FIG. 8a

Pleospora infectoria var. *nigriseda* Rehm, Monit. Jard. bot. Tiflis **25**: 13. 1913.

Perithecia 150–175 μ in diameter, scattered on grayish areas of the stem, smooth, ostiole punctate. Asci broad clavate, 60–75 × 19–21.5 μ. Spores 16–21.5 × 7–9 μ.

Collection: *41*, on *Juncus*, from the Caucasus.

The original description of *Pleospora infectoria* var. *nigriseda* Rehm gives the spores as being the same as those of *P. infectoria,* but the type collection has dark red-brown spores which are mostly 4-septate, although the general form is similar to those of *P. infectoria.*

This species differs from the two preceding in the darker red-brown spores which have more broadly rounded ends, and from the next species in the smaller spores.

Pleospora Clementsii sp. nov. FIG. 7

Perithecia 200–300 μ diametro, globosa vel paululo depressa; pariete 10–20 μ crasso.

Asci clavati, 70–90 μ longi, 17–25 μ crassi, crasse membranacei, basi unguiculiformes.

Sporae biseriatae, ellipsoidales vel clavati-ellipsoidales, brunneae, 23–32 μ longae, 10–14 μ crassae, 4-septatae, interdum 5-septatae, ad septa constrictae, utrinque late rotundatae, saepe sursum latae, deorsum angustatae; septis verticalibus in cellulis centralibus, interdum in cellulis terminalibus.

Specimen typicum (n. *43*) vide infra in enumeratione locorum. In caulibus *Touteriae multicaulis,* in republica Coloradensi.

Perithecia scattered, spheric or slightly depressed, 200–300 μ in diameter, wall smooth, 10–20 μ thick.

Asci clavate, thick-walled, base claw-like, 70–90 × 17–25 μ.

Spores biseriate, ellipsoid to clavate-ellipsoid, dark yellow-brown, 4-septate with one septum above and two below the central primary septum or, occasionally, 5-septate, constricted at the septa, vertical septa in the central cells or sometimes in the end cells, ends broadly rounded, often broader above and tapered below, 23–32 × 10–14(–16) μ.

Collections: *43* (type) and *82,* on *Touteria* and *Mentha,* from Colorado.

These two collections, made by Clements in Colorado, are no doubt the same species, and very similar to *P. dichromatrica,* differing chiefly in the large spores. The large spores seen on the surface of the type collection of *P. dichromatica* bridge this gap between the two species. The collection (*82*), on *Mentha,* bears two species of *Pleospora,* one of which is *P. media* and the other this species. Many of the spores of this latter species are 5-septate which gives the impression of *P. vulgaris* or *P. richtophensis* with spores in which one of the septa in the upper half has failed to

form. The collection (*43*) on *Touteria* (FIG. 7b), has somewhat larger spores.

PLEOSPORA INTERMEDIA Speg. Fungi Chilensis 88. 1910. FIG. 9

Perithecia 200–250 μ in diameter, somewhat flattened spheric, scattered, immersed, then erumpent as small papillate ostioles; walls 10–20 μ thick, parenchymatic.

Asci cylindric-clavate, 70(–120) × 10–12 μ, wall thickened, base claw-like, interspersed with interthecial strips.

Spores biseriate, fusoid to clavate, dark yellow-brown to red-brown, mostly 5-septate, but commonly with one or two septa above and two or three septa below the central primary septum, and then 4- or 6-septate (rarely 7-septate), mostly straight, or slightly inequilateral, asymmetric, commonly rounded above and tapered below, slightly or not at all constricted at the septa, usually with a vertical wall in one or two of the central cells, 19.5–26.5 × 7–8 μ.

Collection: *44* (type) on *Asteriscium*, from Chile.

The spores of this species are often 5-septate, but, probably because of the asymmetric clavate form, they tend to have an extra septum in the lower end, differing in these respects from *P. vagans*. In form the spores resemble those of *P. clavispora*, on *Sorbus* (FIG. 4), which have fewer septa, and those of *P. Ilicis* (FIG. 12), which differ in being fusoid rather than clavate.

PLEOSPORA ORBICULARIS Auersw. Hedw. **7**: 184 and Oesterr. bot. Zeitschr. **9**: 274. 1868. FIG. 10

Pleospora Berberidis Rab. in Fung. Eur. 1727.

Pleospora coluteicola Frag. Fung. Hort. Bot. Matrit. 15. (Trab. Mus. Nac. Cienc. Nat., ser. Bot. **12**.) 1917.

Pleospora herbarum forma *Halimodendri* Frag. Fung. Hort. Bot. Matrit. 18. (Trab. Mus. Nac. Cienc. Nat., ser. Bot. **12**.) 1917.

Perithecia 150–400 μ in diameter, flattened-spheric, immersed beneath the periderm which is often blackened as small spots about the ostiole; wall thin, parenchymatous, sometimes with a slight soft tomentum.

Asci stout-clavate, rather thick-walled and with a claw-like base, 80–125 × 21–35 μ.

Spores biseriate, ellipsoid to mostly clavate, mostly 6-septate, with two septa above and three below the central primary septum, yellow-brown to red-brown, usually asymmetric, broader and rounded above, narrower and tapered below, straight to inequilat-

eral or curved, constricted at the central primary septum, slightly or not at all at the other septa, with vulgaris type vertical septa in the two central primary cells and vertical septa commonly in the upper end cell and lower penultimate cell, 21–36 × 10–16 μ. Some spores are found with four or five septa. These are more abundant in some collections than others.

Collections: *45, 46, 47, 48, 49, 498, 503,* on *Berberis, Allium, Colutea* and *Halimodendron.*

This species characteristically occurs on *Berberis,* but collections on other hosts have been found which appear to be the same species. Winter (7) states that he compared the type of *P. orbicularis* with Fung. Eur. N. *1722,* of *P. Berberidis* and found them to be the same. Berlese (1) repeats Winter's description, but later (2), on the basis of a Celloti specimen, he gives the spores as 20–22 × 7–8 μ and figures (2: *Pl. 17, fig. 2*) 5- to 6-septate spores of the *P. vagans* or *P. dura* type, which is incorrect. Collection No. *45* is given as *Pleospora oligotricha* and as on *Allium,* but it is this species with rather small spores (FIG. 10b). The type (*503*) collection of *Pleospora coluteicola* Frag., first sent to the writer by Dr. Urries, showed nothing but the *Phoma* reported by Fragoso as associated with this species. A second sending yielded a *Pleospora* belonging to this species. Fragoso's original description, however, gives the spores as hyaline at first, then olive-brown, 3-septate and 18–22 × 9–11 μ. Either Fragoso had very immature material of this species or some other species of *Pleospora* no longer present on the type. The name is placed here doubtfully as a synonym. The type collection of *P. herbarum* forma *Halimodendri* Frag. (*498*) has the same clavate 6-septate spores (FIG. 10c), but more of them show seven septa, in which condition they resemble *P. herbarum.* These last two collections also have somewhat larger spores (30–36 × 12–16 μ).

PLEOSPORA TREVOICOLA Speg. Fung. Chilenses 92. 1910. FIG. 11

Pleospora herbarum forma *Genistae* Frag. Fung. Hort. Bot. Matrit. 18. (Trab. Mus. Nac. Cienc. Nat., ser. Bot. 12.) 1917.

Perithecia 100–300 μ in diameter, somewhat flattened-spheric, immersed or erumpent-superficial, on surface as circular blackened spots, with a light colored central area in some cases.

Asci clavate, base claw-like, wall thickened, 60–97(–140) × 17–23(–30) μ.

Spores biseriate, fusoid, oblong ellipsoid or clavate, 5- to 7-septate, yellow-brown to red-brown, straight to slightly inequilateral or curved, symmetric or asymmetric with a broader shorter upper portion, ends rounded or bluntly tapered, constricted at the central septum, the two central cells usually with a vulgaris-type septum, commonly a third (secondary) septum in the lower end, sometimes also in the upper half, vertical septa occasionally in the end cells, 19.5–37 × 7–13 μ.

Collections: *347a, 490* (type), *501,* on *Arenaria, Genista* and *Trevoa.*

These three collections are placed under this species somewhat arbitrarily because they all show the same type of extremely variable spores. This condition is difficult to interpret. It suggests a heterokaryotic condition of the mycelium, for the spores appear to bridge the gap between *P. orbicularis* in the asymmetric series and *P. punctata* or *P. tomentosa* in the symmetric series.

Each of the three collections shows three general kinds of ascospores, usually within the same perithecium. Some spores are symmetric and 5-septate, much as those of *P. richtophensis* (FIG. 11b). Others are either fusoid or clavate with a broad rounded upper portion and have an additional septum in the lower end and resemble the spores of *P. orbicularis* (FIG. 11c). Still others are fusoid, 7-septate (with an extra septum in each end) (FIG. 11a) and resemble the spores of *P. punctata*, etc. Certain collections of *P. media* var. *acuta* (i.e., No. *321*) are very similar to these collections, differing only in the presence of vertical walls in the end cells of a greater number of spores.

The spores of the three collections vary a great deal in size, but this range is about the same as that of *P. orbicularis*. The type collection of *P. trevoicola* is on disintegrated bark covered largely by pycnidia of a *Sphaeropsis* and a *Diplodia*, and Spegazzini's perithecial measurements of 150–180 μ must be confused with these fruit bodies. The spores in the few perithecia seen were old, collapsed and agglutinated. They were small (19.5–25 × 9.5–11 μ) and dark red-brown. The type (*501*) of *P. herbarum* f. *Genistae* has somewhat larger (24–28 × 9–10 μ) spores (FIG. 11c) which are yellow-brown in color. Collection No. *347a* (FIG. 11a) has

much larger spores (33–37 × 11–13 μ) but shows the same variation in form and septation.

In the following group of species, which have ascospores that are more acutely tapered, there is a great deal of variability in spore septation and it is often difficult to circumscribe species. This is due to the fact that as the spore increases in size with maturity, extra septa are laid down in both ends of the spore, often in an irregular fashion. A further complication is the occurrence of vulgaris septation in the central cells of these spores. As a result, a given collection may show spores with varying septation, some simulating species of the asymmetric series with fewer septa, and others simulating corresponding ones of the symmetric series with additional septa. It is necessary, in each collection, to consider the most mature spores as well as the commonest type of septation and the range of variation.

There are a number of such collections which fall within the range of Niessl's three species, *P. coronata, P. dura* and *P. setigera.* The spores of these collections vary in the number of their septa from four to nine, often within a single collection. The descriptions in the literature reflect this situation, and it is next to impossible to fix these species names without type material, which, it seems, Niessl did not leave us. The tabulation, below, of the characters given for these species by Niessl and Berlese, from an examination of Niessl's material, illustrates this situation.

	Perithecia	*Asci*	*Spores*
P. coronata			
by Niessl	fibrillose & penicillate	60–100 × 13–18	22–27 × 7–9 (6- to 8-septate)
by Berlese		120–150 × 15–17	24–30 × 6–9 (6-septate)
P. dura			
by Niessl	fibrillose	120–150 × 15–17	24–30 × 8–9 (7- to 9-septate)
		100–120 × 12–14	32–34 × 8–9 (7- to 8- or 9-septate)
P. setigera			
by Niessl	fibrillose & setose	90–120 × 14–15	22–30 × 8–10 (4- to 5-septate)
by Berlese		70–80 × 8–9	20–24 × 8–9 (4- to 5-septate)

Niessl used the penicillate ostiole to distinguish *P. coronata.* It is doubtful whether this is a constant character. It was seen only

in two collections of this series, which were No. *44*, of *Pleospora coronata*, and the type of *Pyrenophora antarctica* (No. *426*), and seems to be a character of young perithecia rather than any one species. The epithet *coronata* is used, rather arbitrarily, here for a group of collections with narrow, curved ascospores which are mostly 6-septate and do not show any of the vulgaris type of septation in their central cells, whereas *P. dura* is used for those collections with larger, straighter spore types with variable septation (4 to 9 septa) and often with the vulgaris type of septation in the central cells. *P. Ilicis* is a species on woody substrata which has broader spores than *P. coronata* and they are more commonly 5-septate. It represents a variation of *P. vagans*, with an extra septum in the lower end of the spore.

Pleospora Ilicis sp. nov. FIG. 12

Perithecia 300–500 μ diametro, globosa, pustulata, erumpentia; ostiolo papillato; pariete 50–70 μ crasso, intus hyalino, extus atri-brunneo.

Asci clavati, 85–110 μ longi, 12.5–14 μ crassi, paululo crasse membrancei.

Sporae biseriatae vel imbricati-biseriatae, fusiformes, 23–26 μ longae, 7–8 μ crassae, 5- vel 6-septatae, rectae vel modice inequilaterales, plerumque symmetricae, vel infra angustatae, ad septa constrictae, cum septis verticalibus in cellulis centralibus, non in cellulis terminalibus.

Specimen typicum (n. *33*) vide infra in enumeratione locorum. In caulibus *Ilicis paraguayensis*, ex republica Argentinensi.

Perithecia 300–500 μ in diameter, globose, appearing on the surface as thickly scattered pustulate swellings, finally rupturing the periderm; ostioles papillate or short conic; walls parenchymatic, 50–70 μ thick with an outer black-brown and an inner hyaline layer.

Asci clavate, wall slightly thickened, 85–110 × 12.5–14 μ.

Spores biseriate to overlapping uniseriate, fusoid, yellow-brown, 5- or 6-septate, with an additional septum in the lower end, mostly straight or very slightly inequilateral, mostly symmetric, or slightly more tapered below, constricted at the central septum, usually with a vertical septum in one or several of the central cells, 23–26(–32) × 7–8 μ.

Collection: *33* (type), on *Ilex*, from Argentina.

There are apparently two syntype collections of *Leptosphaeria Yerbae* Speg., in the La Plata Museum; No. *2377* yielded the *Leptosphaeria* as described, whereas No. *2378* yielded a dothideaceous *Macrophoma* and the above *Pleospora*. The spores are like

those of *P. vagans,* but commonly show an extra (sixth) septum in the lower end. The spores are broader and straighter than those placed under *P. coronata.*

PLEOSPORA CORONATA Niessl, Notiz. Pyr. (Verh. nat. Ver. Brünn **14**: 176.) 1876. FIG. 13

Perithecia 200–400 μ in diameter, globose or somewhat flattened above, sometimes collapsing pezizoid, thickly scattered, immersed then erumpent as small black spots, sometimes tomentose about the base; walls rather thick (10–50 μ), parenchymatic.

Asci clavate with a somewhat tapering base and a claw-like attachment, wall not much thickened, 65–80 × 8.5–12.5 μ, with scattered interthecial elements.

Spores biseriate above, uniseriate below, yellow-brown, to dark yellow-brown, fusoid, 5-septate at first but with an additional septum in either the lower or both end cells, giving a 6- or 7-septate spore, somewhat broader above the middle and longer tapered below, third (or fourth) cell usually somewhat enlarged, vertical walls in one or more central cells, 19.5–26 × 4.5–6.5 μ.

Collections: *120, 421,* on *Carduus* and *Salsola,* from Italy and North Dakota.

These collections are placed in *P. coronata* even though they do not always show a penicillate ostiole. The spores are first 5-septate but may become 6- or 7-septate by the insertion of a tertiary septum in one or both ends. They differ from *P. Ilicis* in being narrower and more inequilateral, and from *P. dura* in being narrower, having fewer septa, in general, lacking any vulgaris-type septations and in having the central cells more commonly swollen. The perithecia in collection No. *120* soon become exposed and superficial and resemble those of *Teichospora.*

PLEOSPORA DURA Niessl, Notiz. Pyr. (Verh. nat. Ver. Brünn **14**: 178.) 1876. FIG. 14

Pleospora Niessleana Kze. in Kze. Fung. sel. exs. *71.* 1875.

Perithecia 250–350 × 150–250 μ, usually somewhat flattened-globose, thickly scattered, appearing on the surface as circular, blackened flat or sunken spots with a minute central erumpent ostiole, smooth or with a few tomentose hyphae; wall 10–20 μ thick, of small-celled parenchyma.

Asci clavate, tapered toward the base and with a claw-like attachment, wall not much thickened, or slightly so at the apex, 70–90 × 12.5–16 μ, interspersed with interthecial strips.

Spores biseriate above, uniseriate below, fusoid, to clavate-fusoid, (4–)5–7(–9)-septate, yellow-brown to dark yellow-brown, mostly straight or slightly inequilateral, mostly asymmetric, more tapered below, constricted at the primary septum, vertical septa in one or several of the central cells, one to three septa above and two to four below the primary central septum, 20–26(–30) × 7–9 μ.

Collection: *121, 122, 161* (co-type): On *Melilotus* and *Linaria*, from Central Europe.

The spores of these collections show a great deal of variation in the number of septa, even within the same collection. Petrak (3: 218) has reported a similar wide range of variation in spore form and septation for this species, but judging from his spore measurements he has included collections which would be placed in other species of this and also the vulgaris series, as here conceived. In No. *122* there are a number of spores (FIG. 14c) with only one septum above and two below the primary central septum (4-septate). Other spores, in this and other collections, have two or three septa in the upper and two, three or sometimes four septa in the lower portion of the spore, resulting in 5-, 8-, or even 9-septate spores. Collection No. *161*, which is issued in Kunze's Fung. sel. exs. No. *71*, as *P. Niessleana*, and is one of Niessl's co-types, shows the larger spores (FIG. 14a) with more numerous septa. The spores of this species often show vulgaris-type secondary septa in the central cells.

The writer has seen three collections with spores having more than nine septa. There are certain differences between these three collections, but the spores of all three have the same general plan of septation. There is so much variation among the spores of any one collection that they should be united, at least until more material can be studied.

PLEOSPORA ABSCONDITA Sacc. & Roum. in Rel. Libert., ser. 2, No. *157*. (Rev. myc. **1881**: 46.) FIG. 16

Perithecia scattered on purplish or blackened areas of the stem, flattened-spheric, 400–500 × 250–300 μ, causing small pustules on the surface; wall rather thin, 10–30 μ thick, of small-celled parenchyma.

Asci long-clavate with a somewhat tapered base and a claw-like attachment; wall somewhat thickened, 90–160 × 15–23 μ.

Spores biseriate, fusoid or clavate-fusoid, yellow-brown to dark yellow-brown, 6–12(–13)-septate, straight or slightly inequilateral or curved, asymmetric, upper end rounded at first, later tapered but broader than the lower more tapered half, two to three septa above and three to four (or five) septa below the primary septum, and later with vulgaris-type septa in two or four central cells, one, rarely two vertical septa in several central cells, not in the end cells, constricted at the central septum, scarcely so at the other septa, 28–48 × 7–11 μ.

Collections: *162, 350, 543a, 601,* on *Phragmites, Aconitum* and *Silene,* from Switzerland, Tyrol and Washington.

FIGS. 15–18. Ascospores of *Pleospora abscondita* Sacc. & Roum. 15. Spores from collection No. *601.* 16. From collection *350.* 17. From collection No. *162.* 18. From collection No. *543a.*

The use of this binomial for these collections is open to question. The original description gives the spores as 7-septate, but Berlese (2: *Pl. 19, fig. 1*) figures spores, taken from Saccardo's original collection, as 7- to 9-septate and with vulgaris-type septa in the central cells. Roumeguère's Fung. Gall. No. *1598,* in the Farlow Herbarium, shows only a *Leptosphaeria* with 3-septate spores. Rehm's Asc. No. *1140,* of *P. abscondita,* in the Farlow Herbarium (*601*), yielded spores (FIG. 15) which were only 5-septate and showed few vertical septa. This may be a distinct *Leptosphaeria,* but it has the same large perithecia as *P. abscondita* and may be an immature condition of that species. This same exsiccatus, in the Riksmuseet, yielded a few spores with five to seven septa, but

most of them showed nine or more septa. Collection No. *543a* (FIG. 18) consisted only of numerous spores found on the surface of stems from which only *P. comata* perithecia were taken. These scattered spores were 10–12- (or 14-) septate.

The species as represented by these collections seems to have immature spores which are 5-septate and without vertical septa; in more mature spores there are basically two or three septa above and three or four, or rarely five, below the central primary septum. In addition, secondary septa of the vulgaris-type are laid down in two or four of the central cells in fully mature spores.

Collections *350* (FIG. 16) and *543a* (FIG. 18) show the same general plan of septation but the spores of *543a* are somewhat larger. Collection No. *162* has spores (FIG. 17) which are more commonly found with a symmetric septation than in the other two collections (i.e., with four or five septa on each side of the central one).

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33. *Leptosphaeria Yerbae* Speg., on *Ilex paraguayensis*, Missiones, San Pedro, Argentina, Feb. 1907 (La Plata Mus. *2378*) (Type).
37. *Pleospora oblongata* Niessl, on *Cirsium*, Straza bei Velder, July 1891 (Riksmuseet: ex Herb. Rehm).
36. *Pleospora oblongata* Niessl, on *Galium Mollugo*, July 1875 (Riksmuseet: ex Herb Rehm).
38. *Pleospora oblongispora* Rehm, on *Larix decidua*, Mieringen, Schweiz, Aug. 7, 1905, leg. O. Jaap. (Riksmuseet: Herb. Rehm) (Type).
39. *Pleospora oblongata* Niessl, on herbaceous stems, Sulden Gletscher im Ortler, July 1889, leg. Rehm (Riksmuseet: Rehm Herb.).
40. *Pleospora oblongata* Niessl, on herbaceous stem, May 1900, leg. Rehm (Riksmuseet: ex Herb. Rehm).
41. *Pleospora infectoria* var. *nigriseda* Rehm, on *Juncus Gerardi*, in monte Kalmas, Prov. Baku, distr. Dzevat, May 1912, leg. Woronin (Riksmuseet: ex Herb. Rehm *297a*) (Type of var.).
42. *Pleospora kansensis* E. & E., on *Melilotus alba*, Rooks Co., Kans., March 12, 1902, leg. E. Bartholomew (N.Y.B.G.: Fungi Col. *1936*) (Type).
43. *Pleospora herbarum* (Fr.) Rab., on *Touteria multicaulis*, Sulphur Springs, Colo., July 23, 1907 (Farl. Herb., Clem. Cr. Form. Colo. *442*).
44. *Pleospora intermedia* Speg., on *Astericium chilense*, Concepcion, Chile, Jan. 1909 (La Plata Mus. No. *2169*) (Type).

45. *Pleospora oligotricha* Niessl, on *Allium* sp., bei Insel, June 4, 1942, leg. K. H. (Richmeyer) (Riksmuseet: ex Herb. Mus. H. Nat. Vindob.).
46. *Pleospora Berberidis* Kze., on *Berberis vulgaris,* Rollsdorf (Sachsen), June, leg. J. Kunze, Winter No. *3750* (Riksmuseet, Rab. Fung. Eur. *1722*) (Type).
47. *Pleospora orbicularis* Awd., on *Berberis vulgaris,* Leutschau (Zips) Ungarn, Aug. 1888, leg. Bresadola (Riksmuseet: ex Herb. Bresadola *259*).
48. *Pleospora Berberidis* Kze., on *Berberis,* Tyrol, Aug. 1872, leg. Rehm (Riksmuseet: ex Rehm Herb.).
49. *Pleospora orbicularis* Awd., on *Berberis,* Waldrand bei Neufriedenheim, München, March 1902, leg. Rehm (Riksmuseet, Rehm Asc. *1439*).
57. *Pyrenophora Scdi* Roum. & Brun., on *Sedum alba,* Gallia, Saintes, Charents-inferieure, March 1885, leg. P. Brunaud (Riksmuseet: ex Herb. Sydow) (Type).
70. *Pleospora Tragacanthae* Rab., on *Phaca frigida,* Furstenalp, Graubunden, 1800 m., Aug. 15, 1903, leg. A. Volkart (Riksmuseet: Rehm Asc. *1566*).
82. *Pyrenophora phacocomoides* Sacc., on *Mentha penarda,* Boulder, Colo., July 29, 1907 (Farl. Herb., Clem. Cr. Form. Colo. *452,* pr. p.).
120. *Pleospora Lecanora* (Fabre) Rehm, on *Salsola Tragus,* Kulm, N. Dak., July 19, 1913, leg. J. Brenckle (Riksmuseet: ex Rehm Herb.: ex Brenckle Herb., Fung. Dak. *240*).
121. *Pleospora dura* Niessl, on *Melilotus officinalis,* Mähr-Weisskirchen, Aug. 22, 1922, leg. Petrak (Riksmuseet, Petr. Fl. Boh. & Mor. *1875*).
122. *Pleospora dura* Niessl, on *Linaria genistifolia,* Brünn in Mähren, Aug. 1927, leg. J. Hruby (Riksmuseet, Petr. Fl. Boh. & Mor. *2438*).
160. *Pleospora Galii* Romell inedit., on *Galium verum,* Sdl. par Huddinge: Huddinge, April 29, 1894, leg. Romell (M.A. *3689*) (Riksmuseet: ex Herb. L. Romell *16094*).
161. *Pleospora Niesslcana* Kze., on *Melilotus alba,* Oberreisdorfer Thal pr. Islebiam (Saxony), May 1875, leg. J. Kunze (Riksmuseet, Kze. Fung. sel. 71) (Type).
162. *Pleospora dura* Niessl, on *Aconitum,* Kartel, Gletscher, Morsthal (Tyrol), July 1878, leg. Britzelmayer (Riksmuseet: ex Herb. Rehm).
334. (*Pleospora?*), on *Galium Mollugo,* Gottland: Bro, June 18, 1920, leg. T. Vestergren (Riksmuseet: Fung. Suec.).
337. (*Pleospora?*), on *Sorbus Aucuparia* bark, Bromma, Drottningsholmsvagert, Nov. 21, 1920, leg. T. Vestergren (Riksmuseet).
347. (*Pleospora?*), on *Arenaria uintahensis,* Wasatch Mts., Salt Lake City, Utah, June 7, 1904, leg. A. O. Garrett (Riksmuseet: ex Herb. Sydow).

347a. Same data; second fungus.

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421. *Pleospora coronata* Niessl, on *Carduus sphaerocephalus,* Italy: Conegliano, Aug. 1878 (La Plata Mus. No. *7185*).

426. *Pyrenophora antarctica* Speg., on *Azorella trifurcata*, Gregory Bay, Patagonia, June 1882 (La Plata Mus. No. *2198*) (Type?).
427. *Pyrenophora dichromatricha* Speg., on *Euphorbia terracina*, Puerto Luz, Las Palmas, Dec. 1913 (La Plata Mus. *2199*) (Type).
490. *Pleospora trevoicola* Speg., on *Trevoa trinervis*, Chile: Santiago, colinas de San Bernardo (La Plata Mus. No. *2151*) (Type).
498. *Pleospora herbarum* f. *Halimodendri* Frag., on *Halimodendron argenteum*, prope Matritis, in Hort. Bot., Aug. 1916, leg. Caballero (Herb. Jard. Bot. Madrid: Fung. *2101*) (Type).
501. *Pleospora herbarum* f. *Genistae* Frag., on *Genista iberica*, Madrid, in Bot. Gard., Aug. 26, 1916, leg. Caballero (Herb. Jard. Bot. Madrid: Fung. *2121*) (Type of form).
503. *Pleospora coluteicola* Frag., on *Colutea tragacanthoides*, prope Matritis, in Hort. Bot., Aug. 26, 1916 (Herb. Jard. Bot. Madrid: Fung. *2178*) (Type).
543. *Pleospora comata* Niessl, on *Silene Macounii*, Frozen Lake, Mt. Rainier, Wash., 6,700 ft., Aug. 17, 1948, leg. E. G. Simmons No. *1969* (Wehm. Herb.).
543a. Same data, second fungus (*Pleospora abscondita* Sacc. & Roum.).
601. *Pleospora abscondita* Sacc. & Roum., on *Phragmites*, Ufer des Bodensee bei Kreuzlingen (Shweiz), Oct. 1892, leg. Wegelin (Farl. Herb., Rehm Asc. *1140*).
776. Undetermined, on *Barbaraea stricta*, Suecia: Storlien, Jemtlandiae, June 22, 1932, leg. A. G. Eliasson (Riksmuseet: Fl. Suec.).

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THE CONNECTION BETWEEN BOTRYOTINIA FUCKELIANA AND BOTRYTIS CINEREA [1]

J. Walton Groves [2] and Constance A. Loveland [3]

(with 4 figures)

The successful production of apothecia in culture from isolates of *Botrytis* species of the *cinerea* type was reported by Groves and Drayton (1939). The apothecia belong to the genus *Botryotinia* Whetz. in the family Sclerotiniaceae Whetz. (1945). Although de Bary (1866) claimed that *Botrytis cinerea* and a discomycete, which he named *Peziza fuckeliana*, were different states of the same fungus, no attempt was made by Groves and Drayton to name their fungus because the exact identity of de Bary's *Peziza fuckeliana* was in doubt.

Differences of opinion arose in mycological literature concerning the connection between *Botrytis cinerea* and *Peziza fuckeliana*. The literature on this point has been well reviewed by Buchwald (1949). He came to the conclusion that the weight of evidence favoured the view that *Peziza fuckeliana* had a *Botrytis* of the *cinerea* type as its conidial state, but he felt that it was probably retricted to *Vitis* as the host plant. It now seems possible to provide conclusive evidence for the connection between the two states and also that the fungus is not restricted to *Vitis*.

Gregory (1949) reported the finding of slides of de Bary's original material of *Peziza fuckeliana* in the British Museum in excellent condition. He was able to make a study of this material and published notes and photographs together with a full discussion of the literature pertaining to the connection between these two states. Dried material of the apothecia produced in our cultures

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was sent to Gregory who very kindly made an examination of it and reported (*in litt.*) that it appeared to be identical with *Peziza fuckeliana.*

In addition to this morphological evidence, certain cultural evidence as to the identity of our material with that of de Bary is now available. *Peziza fuckeliana* was originally described as occurring on *Vitis* in the Rhine valley. Since apothecia of many of the Sclerotiniaceae are very similar morphologically, it was considered necessary to establish that matings were possible between our single ascospore cultures and strains of *Botrytis cinerea* from the type host and as closely as possible from the type locality.

In the original experiment in which the apothecia were first obtained, they were developed in nine isolates originating from apples, potatoes, and celery (FIG. 1). By the use of single ascospore cultures, attempts were then made to determine the type of mating behaviour in each of these nine isolates.

The technique used was essentially the same as that described by Drayton (1937) with slight modifications. The usual experimental schedule was as follows:

1. The fungus was planted on Petri dishes of potato dextrose agar and grown at room temperature for two to four days.

2. Freshly growing mycelium was transferred to wheat plates. These were prepared by adding 8 gms. of wheat and 25 cc. of water to each Petri dish and sterilizing in the autoclave. A soft wheat, such as Dawson's Golden Chaff, proved to be the most satisfactory for preparing the wheat plates.

3. After two or three days at room temperature the wheat plates were then placed at 5° C or 14° C in the dark for a period of four to six weeks or until the sclerotia were well developed.

4. The sclerotia were then removed from the wheat plates with a pair of fine, sterile forceps and placed on moist, sterile, quartz sand in 100 mm. preparation dishes. The dishes were then placed in the dark at 0° C for at least one month.

5. The sand cultures were transferred to 5° C in the dark for two weeks.

6. Spermatization was carried out and the sand cultures moved to 14° C for one month.

7. The dishes were placed in the greenhouse exposed to north light and shaded from direct sunlight. The greenhouse temperature was kept at about 10–14° C as closely as possible, and sterile water was added to the dishes from time to time to keep the sand moist.

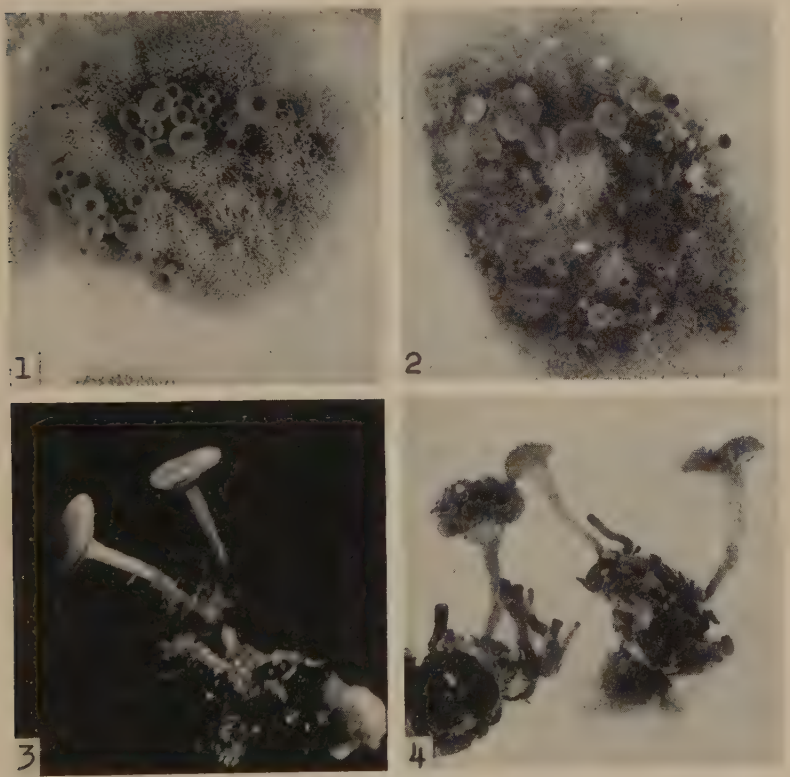

FIG. 1. Apothecia of *Botryotinia fuckeliana* developed in culture, × 1. 2. Apothecia of *B. fuckeliana* from a cross between a single ascospore culture and a Swiss isolate from *Vitis*, × 1. 3. Two apothecia enlarged, × 2 approx. 4. Abnormal multiple head development believed to be related to high temperatures in the greenhouse, × 2.

It was found that better results were obtained when the sclerotia were covered with a thin layer of moist sterilized soil before the dishes were placed in the greenhouse. Sometimes this was done at the same time as the spermatization by adding the spermatial suspension to sterilized soil which was then placed on the group

of sclerotia and distributed over them with a sterilized camel's hair brush. Equally satisfactory results were obtained by painting the suspension on to the sclerotia directly, and adding the soil later before they were moved to the greenhouse.

At first, in an effort to conserve space and glassware, sclerotia from several isolates were spaced some distance apart on the sand in the same dish, but, from the inconclusive results obtained, it was soon realized that it was possible for the spermatia to be washed from one isolate to the other, and that it was absolutely essential to keep each isolate in a separate dish throughout the entire experiment.

TABLE I

CROSSES BETWEEN SINGLE ASCOSPORES OF B56 FROM CELERY

♀ \ ♂	CK	SS_2	SS_5	SS_{24}
SS_1	−	−	+	−
SS_2	−	−	+	−
SS_5	−	+	−	+
SS_9	−	+	−	+
SS_{20}	−	−	+	−
SS_{21}	−	−	+	−
SS_{22}	−	−	+	−
SS_{23}	−	−	+	−

From these experiments it was established that the single ascospore cultures exhibit the bipolar type of mating behaviour similar to that found in *Stromatinia gladioli* (Drayt.) Whetz. by Drayton (1934). The results of one experiment with cultures from single ascospores obtained from apothecia of isolate B56, originally isolated from celery in storage, are illustrated in TABLE I.

The appearance of apothecial fundaments was considered to be sufficient evidence that fertilization had occurred, for no fundaments were ever observed in dishes containing unspermatized or self-spermatized sclerotia or as the result of a theoretically infertile cross. Usually the apothecia matured, but in certain in-

stances they failed to complete their development for a number of reasons. An adverse effect was noted as a result of the drying of the sand in the dishes, or when they were subjected to sudden drops or undue rises in temperature. Under these conditions the apothecial fundaments turned black and further development ceased. Another abnormality that was observed, that appeared to be related to high temperatures in the greenhouse, was the production of several smaller discs on one stipe instead of the usual single disc (FIG. 4).

After the initial experiments that led to the development of apothecia, successful crosses were made between single ascospore cultures of the different isolates. The results of such crosses between the isolates B56 and B71, from celery and potato stalks respectively, are given in TABLE II.

TABLE II

CROSSES BETWEEN SINGLE ASCOSPORES OF B56 FROM CELERY AND B71 FROM POTATO

♀ \ ♂	$B_{56}SS_2$	$B_{71}SS_3$	$B_{71}SS_4$	$B_{71}SS_9$
$B_{56}SS_5$	+	+	−	+
$B_{56}SS_9$	+	+	−	+
$B_{71}SS_9$	−	−	+	−
$B_{71}SS_{20}$	−	−	+	−

Single ascospores were isolated from the apothecia developed from these crosses and successful crosses were also made between single ascospores from the other isolates. Hence it was concluded that all these isolates belonged to the same species.

It was then considered necessary to prove that crosses were possible between these single ascospore cultures and isolates of *Botrytis cinerea* from *Vitis* collected in the Rhine valley. Due to the war this work had to be abandoned for some years, but the single ascospore cultures were maintained and in 1947 the senior author had the opportunity to visit Zurich, Switzerland where, through the kind cooperation of Dr. L. Ettlinger and Dr. S.

Blumer, it was possible to obtain two isolates of *B. cinerea* from Malans and Waedenswils, Switzerland. Crossing experiments were then undertaken between these two isolates and our single ascospore cultures.

Since the two isolates from *Vitis* were made directly from nature, it was possible that both mating strains were present and, therefore, if they were used as females, it was possible that any apothecia resulting would not be due to fertilization by the single ascospore culture used as a male. This difficulty was overcome by using the Swiss isolates as males, since any apothecia that developed must result from a fertilization of the single ascospore culture by spermatia from the Swiss isolate.

With the isolates from *Vitis* as males, fertilization was effected in five of the single ascospore cultures and single ascospores were isolated from the apothecia that matured (FIGS. 2, 3). It was, therefore, concluded that the isolates from *Vitis* from Switzerland belonged to the same species as the isolates from apples, potatoes, and celery from Canada.

In 1939, samples of a shipment of grapes var. Emperor shipped from California to Moncton, N. B., were received at the Division of Botany and Plant Pathology. These grapes had arrived in a damaged condition and were attacked by *Botrytis cinerea*. Isolations were made from this material and crossing experiments undertaken with our single ascospore cultures. Apothecia were obtained when the California isolates were used as females, but apothecia also appeared in the unspermatized checks and it is believed that the original isolates were a mixture of strains. Ten single ascospore cultures were isolated, but they were unfortunately lost before further tests could be carried out with them. It seems probable that the California isolates also belonged to the same species as the Canadian and Swiss isolates, but this was not completely established.

However, it has been established that (1) isolates of *B. cinerea* produce sclerotinioid apothecia when compatible strains are crossed, (2) single ascospores from these apothecia give rise to typical fructifications of *B. cinerea,* (3) single ascospore cultures are self-sterile and exhibit bipolar mating reaction, (4) isolates of

B. cinerea from *Vitis* from Switzerland are interfertile with Canadian isolates from apples, potatoes, and celery, (5) apothecia from such crosses are morphologically similar to the type of *Botryotinia fuckeliana* (de Bary) Whetz. It is, therefore, concluded that *B. fuckeliana* is established as the perfect state of *B. cinerea.*

TAXONOMY

The genus *Botryotinia* was erected by Whetzel (1945) with *B. convoluta* (Drayt.) Whetz. as the type species. It is known that Professor Whetzel would have liked to designate *Peziza fuckeliana* as the type of *Botryotinia* but refrained from doing so because of the doubt that still existed concerning the connection between it and *Botrytis cinerea.* The choice of *Botryotinia convoluta* does not alter the generic concept in any way since this species and *B. fuckeliana* are very closely related and certainly congeneric.

It should also be pointed out that, although we have been able to show that our isolates from apples, potatoes, and celery will cross with isolates from *Vitis* from Switzerland, and that apothecia resulting from such crosses are morphologically similar to the type of *Peziza fuckeliana,* it does not necessarily follow that all other isolates of *Botrytis* species of the *cinerea* type belong to *Botryotinia fuckeliana.* The fact that isolates from these varied hosts and widely separated geographic localities are interfertile, suggests that the bulk of the isolates of *Botrytis cinerea* will prove to belong to this species. However, it is quite probable that further work will make it possible to recognize specific entities in this great *B. cinerea* complex.

This makes it desirable to fix more accurately the concept of *Botrytis cinerea.* This species was described by Persoon (1801, 1822) and the name was accepted by Fries in the Systema Mycologicum (1832). An amendment to the International Rules of Nomenclature passed at the VII International Botanical Congress stated that in some cases it may be desirable to accept the type of a pre-starting-point author. This would seem to be a case in which it is desirable to do so.

After the 1950 Botanical Congress, the senior author had the opportunity of examining material in the Persoon Herbarium at Leiden. There were three specimens labelled *Botrytis cinerea,* No. *910.262.835,* No. *910.262.845,* and No. *910.262.847.* No. *835* consisted of a couple of pieces of a grass stem and leaves. There were some blackened areas on the leaves but nothing to suggest sclerotia and no *Botrytis* conidiophores or conidia were found. This specimen would appear to be of no value.

No. *845* consisted of fairly abundant material of some herbaceous stem split and glued to the mounting paper. Conidiophores resembling those of *B. cinerea,* as we understand it, are fairly abundant and sclerotia are present which, although small (about 1–2 mm. in length), are typical *Botryotinia* sclerotia firmly attached to the substrate. One mount was made from this material and typical conidiophores were present, and, although spores were not abundant, some were found measuring 8.5–13 × 6–9 μ.

No. *847* consisted of a small piece of unrecognizable plant material about 3 × 1 cm., bearing *Botrytis* conidiophores but no sclerotia. One mount was also made from this specimen and there were abundant spores 10–15 × 7.5–10 μ.

In view of the fact that in No. *845* the material was more abundant and bore both conidia and sclerotia, this specimen should be taken as the type.

It is, therefore, proposed that No. *910.262.845* in the Persoon Herbarium, Leiden, be designated as the lectotype of *Botrytis cinerea* Pers.

A complete description of *Botryotinia fuckeliana* based on our material is included.

BOTRYOTINIA FUCKELIANA (De Bary) Whetz. Mycologia 37: 679. 1945.

Peziza fuckeliana De Bary nom. nud. Morph. u. Physiol. der Pilze, Flechten, und Myxomyceten. p. 30. 1866.

Peziza fuckeliana De Bary in Virch. u. Hotzendorf's Samml. gemeinverst. wiss. Vortr. Ser. IV, Heft 87 & 88: 25. 1869.

Sclerotinia fuckeliana Fuckel, Symb. Mycol. p. 330. 1870.

st. conid.

Botrytis cinerea Pers. Syn. Meth. Fung. p. 690. 1801.
Botrytis cinerea Fries, Syst. Myc. **3**: 396. 1832.
Botrytis fuckeliana Buchw. Den Kgl. Vet.-Landb. Aarsskr. **1949**: 133. 1949.

Sclerotia hard, black, plano-convex and firmly attached to the substrate, very variable in size and shape, rounded to elongated and more or less curved, sometimes convoluted and very irregular, 1–15 mm. in length, sometimes forming confluent masses; in section composed of closely interwoven, hyaline, thick-walled hyphae with a thin, pseudoparenchymatous rind composed of dark brown to black, almost isodiametric cells about 5–10 μ in diameter.

Apothecia arising from the sclerotia singly or in clusters, long stipitate, at first deeply concave or cup-shaped to funnel-shaped, expanding and becoming plane and finally convex, often umbilicate, 1.5–7.0 mm. in diam. and 3–10–(15) mm. in height, the stipe rather stout, 0.5–1.5 mm. in diameter, tapering downwards slightly, the disc avellaneous to wood-brown or verona brown to bister with a delicate, paler margin, the stipe concolorous becoming darker below to blackish at the base, nearly glabrous or minutely pubescent; tissue prosenchymatous, in the stipe composed of hyaline, more or less vertically parallel to slightly interwoven, septate hyphae about 5–10 μ in diameter, at the outside slightly narrower and more compactly arranged and faintly brownish, but scarcely differentiated into a definite excipulum, in the hypothecium the hyphae becoming more intricately interwoven and at the outside a pseudoparenchymatous excipulum is differentiated, composed of large, hyaline, elongated to nearly isodiametric or polygonal cells mostly 10–20 μ in diameter but sometimes up to 60 μ in length, subhymenium differentiated as a narrow, brownish zone of closely interwoven hyphae about 2.5–5.0 μ in diameter; asci cylindric with a long, tapering stalk, eight-spored, (105)–120–140–(160) × 6.5–9.5 μ; ascospores hyaline, one-celled, ellipsoid to ovoid, uniseriate, 9–12 × 4.5–6.0 μ; paraphyses hyaline, filiform, septate, unbranched or very occasionally so, 2.0–2.5 μ in diameter with the tips sometimes slightly swollen.

Conidiophores arising from the mycelium or from the sclerotia, erect, brown, septate, simple or usually branched toward the tip, the tip rounded or slightly swollen, 5–22 μ in diameter, 1–3 mm. in height; conidia grayish-brown in mass, nearly hyaline under the microscope, ellipsoid to ovoid, one-celled, borne on small stalks arising from the tips of the conidiophores and forming botryose clusters, 8–14–(18) × (5)–6–9 μ.

Spermatia hyaline, globose, one-celled, 2.0–2.5 μ in diameter, produced endogenously from hyaline, flask-shaped phialides which may arise singly from the mycelium or more commonly are aggregated into clusters forming spermadochia.

HOST: *Vitis, Pyrus, Solanum, Apium* and probably many other plants.

TYPE: Slide in British Museum, *Peziza fuckeliana* 22/10/64. (Gregory (**1949**)): st. conid. Persoon Herb. *910.262.845.*

SPECIMENS EXAMINED: Apothecia developed in culture, DAOM *19489, 19490, 19491, 19492, 19493, 19494, 19495, 28495, 28496.*

SUMMARY

The connection between *Botryotinia fuckeliana* and *Botrytis cinerea* has been established by cultural and morphological studies and a lectotype of *B. cinerea* has been designated.

ACKNOWLEDGMENTS

The authors are indebted to Dr. P. H. Gregory for his kindness in comparing our material with the type of *Peziza fuckeliana*, to Dr. L. Ettlinger and Dr. S. Blumer for providing cultures of *Botrytis cinerea* from *Vitis*, to Dr. H. J. Lam and Dr. R. A. Maas Geesteranus for permission to examine material in the Persoon Herbarium, and to Dr. F. L. Drayton for his continued interest in the problem and helpful suggestions.

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A NEW GENUS OF THE MUCORALES

S. B. Saksena

(with 50 figures)

The writer has been engaged for the past few years in the study of the fungal flora of some forest soils in the neighborhood of Sagar, in Madhya Pradesh State, India. The fungus described below was observed in cultures of soil from the Patharia forest first in October 1950 and again in April 1952. Only the sporangial stages have been observed.

MATERIAL AND METHODS

The soil from Patharia collected in October was somewhat moist. Samples were taken from a depth of about 4″ with the help of a sterile spatula, packed in sterile containers and brought to the laboratory.

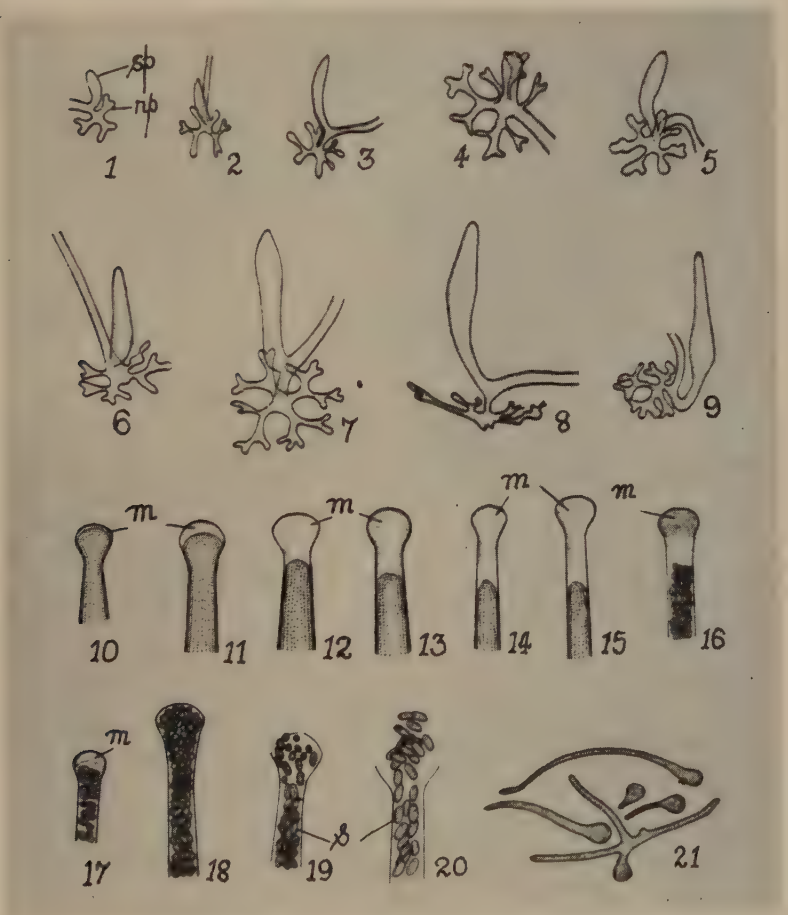
Preliminary isolations were made in Petri dishes with plain agar, Waksman's agar (Waksman **1927**) and potato-dextrose-agar using the dilution technique (Waksman **1927**). The fungus first appeared in Petri dishes with potato-dextrose-agar. Further isolation and purification for purposes of detailed study were made in plain agar and potato-dextrose-agar. Single spore isolations were made by transferring single germinating spores with the help of a stereoscopic microscope.

STRUCTURE OF THE MYCELIUM

The mycelium is fast-growing and covers the entire Petri dish within a week from inoculation. It consists of a large portion of much branched submerged hyphae and profuse flocculent aerial hyphae. The hyphae are 3.2–6.4 μ thick, generally aseptate, but with a few septa in older hyphae. Mycelium treated with chlor-zinc-iodide does not give a reaction for cellulose.

DEVELOPMENT OF SPORANGIUM

The fungus was for a long time sterile and no reproductive structures were seen to develop in it. But when pieces of potato-dextrose-agar with the growing mycelium were floated in sterile distilled water, sporangia were formed abundantly. During the



FIGS. 1–9. Earlier stages in the development of the sporangium. (Sp = Sporangial primordia, rp = rhizoidal primordia.) × 860. FIGS. 10–15. Stages showing the formation of apical portion of neck with mucilage plug (m). × 1370. FIGS. 16–20. Stages showing the gradual dissolution of the apical mucilage (m) and the liberation of spores (s). × 860. FIG. 21. Germinating spores. × 860.

past year and more, sporangia have been observed in solid agar medium only a few times and that sparingly. Hence the formation and development of the sporangium have been followed by floating pieces of agar on which the fungus was growing in sterile water.

The sporangia are generally formed at the extremities of short unbranched hyphae. These fertile hyphae are usually found floating on the surface.

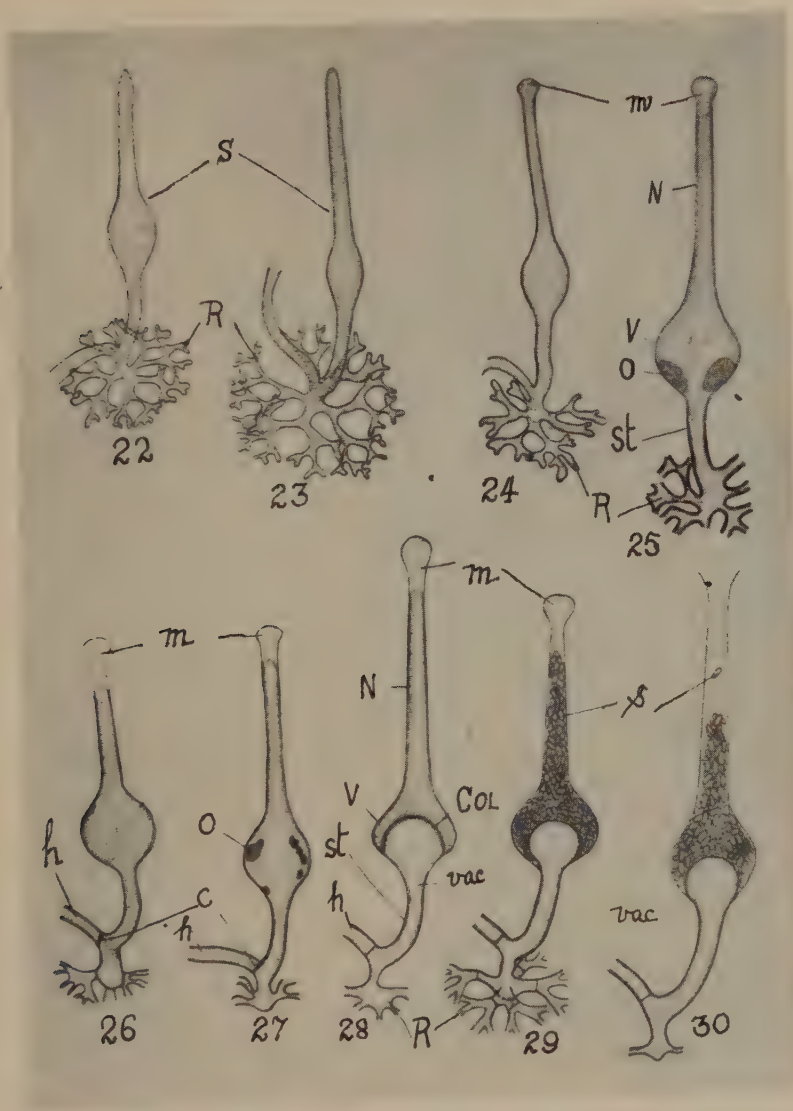
From the end of the sporangium-forming hypha is produced a strongly dichotomously branched rhizoidal complex. A little above the place of origin of the rhizoids a small protuberance is put forth in the opposite direction which ultimately develops into a sporangium (FIGS. 1, 39, Si).

The complex of rhizoidal branches shows some radial symmetry in one plane. These branches are at first aseptate. Later, however, the main branches of the rhizoidal system are each cut off by a septum from the common place of origin (FIGS. 29, 40).

The sporangial primordium (FIGS. 1, 2, 3) at first becomes long and is broader than a normal hypha. The apex at this stage is tapering (FIGS. 39, 40). Later, a portion of this long developing primordium a little above the place of origin slowly swells out into a spherical venter-like structure. The small portion between this venter and the place of origin elongates a little more and becomes the stalk of the sporangium.

Simultaneously with the development of the venter, the upper portion elongates further to form a long neck, the end of which becomes a little bulged out (FIGS. 8, 9, 22–24, 41). The apex becomes rounded on the exterior. Thus the sporangiophore, at this stage, consists of the rhizoidal system below, a stalk, a venter, and a neck with a swollen, somewhat rounded apex. The venter and the neck together give it the appearance of a round-bottomed flask (FIGS. 25–30, 43).

During the increase in size of the sporangium, the contents become very dense. With the increase in the size of the developing sporangium the contents also increase in quantity and keep pace with it, completely filling up the cavity. Later, as the neck elongates further and the tapering apex swells up, the protoplasmic



FIGS. 22–27. Further development of the sporangium (S) with clear differentiation of the rhizoidal complex (R), stalk (St) of the sporangium, venter (V) and the neck (N). × 860. 25, 27. Show oil globules (O). 26, 27. Stages showing the cutting off of the sporangium from the parent hypha (h) by a septum (c). FIG. 28. A fully formed sporangium with a columella (Col.), rhizoidal complex (R), venter (V) and the neck (N). FIGS. 29–30. Formation and liberation of spores from the ripe sporangium. × 1370. (S = spores; vac = vacuoles.)

contents do not increase any more and there is a gradual secretion of a dense mucilaginous substance at the apex, which gives pectic reactions but gives no cellulose reaction with chlor-zinc-iodide (FIGS. 10–15, 42, 44). At maturity the apex of the sporangium is occupied by a dense refractive substance forming as it were a sort of plug (FIGS. 44, 49). At this stage both the sporangium and the rhizoidal complex appear as if occupying the upper and the lower ends respectively of a short vertical hypha, and the parent hypha itself appears as if it were a side branch of this vertical hypha (FIGS. 26–29, 50). This parent hypha is soon cut off by a septum. The protoplasmic contents in the sporangium at this stage are very dense and show large oil globules. Soon a dome-shaped columella is differentiated in the venter. The contents of the columella and the stalk are of a thin consistency and soon large vacuoles appear in them (FIGS. 28, 30).

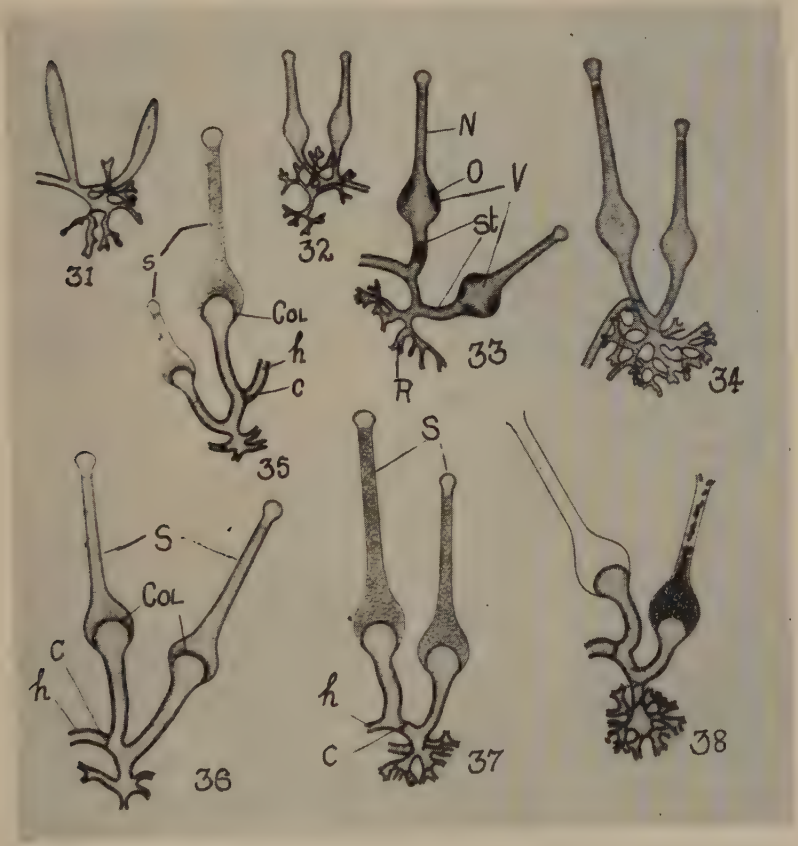
The wall of the sporangium is generally thin, smooth and hyaline and no calcium deposit has been observed. However, the walls of the rhizoidal complex and of the stalk become thickened and somewhat brownish in color.

Usually a single sporangium is developed over a rhizoidal complex at the ends of the sporangia-forming hyphae, but sometimes two sporangia are developed from two primordia formed above a single rhizoidal complex (FIGS. 31–38, 47). These two primordia may be formed either simultaneously or successively. In the former case, the septum cuts off both sporangia from the rest of the original hypha, which therefore lie between the septum and the rhizoidal complex (FIGS. 36, 38). In the latter case the septum is formed between the two sporangia (FIG. 37) and it seems that the sporangium which is proximal to the rhizoids is the older of the two.

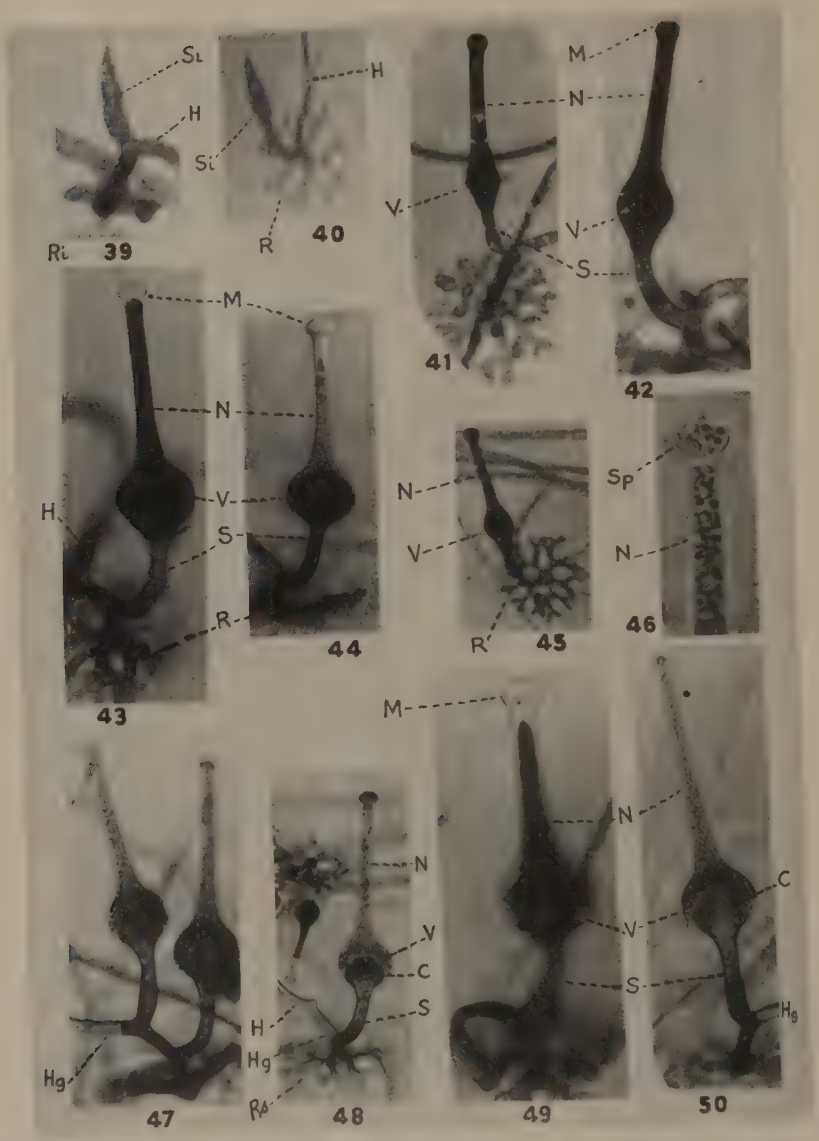
The protoplasm of the venter above the columella is now divided into a number of tiny protoplasts by the formation of cleavage furrows (FIG. 44). The tiny protoplasts become a little bigger, secrete a wall and are thus transformed into spores. These spores come to occupy the entire sporangial cavity (FIGS. 16–18, 48–50). The mucilaginous plug gradually becomes dissolved away and finally the thin wall at the convex apex is also gelatinised. The

spores are then discharged through the dissolved apex of the sporangium (FIGS. 19, 20, 30, 38, 46). The expulsion is brought about by a passive movement of the spores through the neck in a series.

These liberated spores seen to accumulate outside the neck and do not show any movement. Often, some spores were seen to



FIGS. 31–38. Stages in the formation and development of double sporangia. × 860. 31–34. Stages leading to the formation of the sporangium and differentiation of the venter (V) and the neck (N). (O = oil globules; R = rhizoidal complex.) 35–36. Two fully formed sporangia with columella (col) and a septum (c) cutting off both the sporangia (S) from the parental hypha (h). 37. Two fully formed sporangia (s) with a septum (c) formed in between the two sporangia. 38. Showing two sporangia, in one of which the spores are being liberated; the other is empty.



FIGS. 39–43, 45. Formation of the sporangium. 39. Young sporangium initial. 40. Further growth of the sporangium initial. 41. Differentiation of the venter and the neck. 42, 43, 45, 49. Further development of the venter, differentiation of the apex and secretion of the mucilage-like substance. FIGS. 44, 48, 50. Fully-formed sporangia with columella and spores. 44. Formation of distinct protoplasts by the sporangial contents. 48, 50.

remain inside the venter of the sporangium itself and to germinate *in situ*. The spores at the time of liberation are oblong in shape. The liberated spores were transferred to Czapek's liquid medium for germination. During germination the spores swelled and became spherical, sub-spherical or ovoid. Usually one germ tube was produced (FIG. 21).

Sexual reproduction in this fungus has not been observed so far in any of the cultures.

This fungus with its non-septate hyphae and aerial sporangia containing aplanospores clearly belongs to the Mucorales. The presence of a distinct columella and the fact that the sporangia are all of the same shape (i.e., there are no sporangiola or "conidia") and lack heavily cutinized walls indicate a relationship to the family Mucoraceae. On the other hand, the dichotomously branched hyphae (which probably act as props) at the base of the sporangiophores, suggest a kinship to the genus *Syncephalis*, some species of which have remarkably similar structures. The present genus, because of the flask-shaped sporangia, their dehiscence by the dissolution of an apical plug and the method of spore discharge, does not seem to fit into any of the present families of the Mucorales. Its position within the order can best be determined after the sexual reproductive stages have been found.

It is proposed to erect a new genus. The genus is named, *Saksenaea*, after Dr. Ram Kumar Saksena of the Allahabad University, under whose guidance and advice the writer is working. The type species, which at present is the only one known in this new genus, may be called *Saksenaea vasiformis* sp. nov.

Later stages in the formation and liberation of spores; apical plug has been dissolved and only the wall is intact. 46. Apex of a mature sporangium showing the apical opening through which the spores are liberated. 47. Two sporangia formed from the same parent hypha. (*R* = Rhizoid complex; *Rs* = Rhizoidal septum; *Ri* = Rhizoidal initials; *S* = Stalk of the Sporangium; *Si* = Sporangium initial; *V* = Venter; *N* = Neck; *H* = parental hypha; *Hs* = Septa cutting off the parental hypha and the Sporangium; *M* = mucilage-like substance; *C* = Columella; *Sp* = Spores.) (Magnifications: 39, × 650; 40, × 250; 41, × 375; 42 & 49, × 460; 43, × 550; 44, 47, 50, × 500; 45, × 300; 46, × 900; 50, × 350.)

Saksenaea gen. nov.

Sporangia urceolata, ventre sphaerico distincto atque collo longo, insidentia singula vel bina apicibus hypharum aerearum, sporangiophoris singulis supra massam rhizoidalem saepe bifurcatam positis. Columella prominens atque cupulae similis. Sporangia dehiscentia per dissolutionem obstructionis vel operculi apicalis mucilaginosi; sporae dispersae per hoc collum.

Sporangia flask-shaped with a distinct spherical venter and a long neck; formed singly or in pairs at the ends of aerial hyphae, each sporangiophore above a profusely dichotomously branched rhizoidal complex. Columella prominent and dome-shaped. Sporangia dehiscing by the dissolution of an apical mucilaginous plug, spores discharged through this neck.

Saksenaea vasiformis sp. nov.

Mycelium bene evolutum, cito crescens hyphis ramosissimis productis duplicis speciei, quarum una submersa atque profuse ramosa, altera vero flocculenta atque aerea 3.2–6.4 μ lata. Sporangia ut plurimum singula, nonnumquam bina, erecta, insidentia rami hyphalis apici, qui infra structura rhizoidea dichotome ramosa est ornatus, rhizoideis 3.2–4.8 μ, latis, pedicellata, pediculis 6.4–9.6 × 24–64 μ, urceolata ventre sphaerico, 16–43.2 × 22.4–51.2 μ, columella distincta cupulae simili praedita; venter collo longo superposito 6.4–11.2 × 54.4–200 μ ornatus; colli apex paululo latior 8.0–14.4 μ diam., obturamento mucilaginoso occlusus. Sporangiosporae parietibus praeditae, oblongae, 1.4–2.1 × 2.8–4.2 μ, per collum obturamento apicali dissoluto emissae; in germiatione ut plurimum unus germinationis tubulus efformatus.

Typus lectus in terra, in loco Patharia Forest, in Sagar, in regione Madhya Pradesh, in India.

Mycelium well developed, fast-growing with much-branched hyphae of two kinds, one submerged and profusely branched, the other flocculent and aerial, 3.2 to 6.4 μ broad. Sporangia generally single, sometimes in twos, erect, developed at the end of a hyphal branch with a dichotomously branched rhizoidal structure below, rhizoids 3.2 to 4.8 μ broad, stalked, stalk 6.4–9.6 × 24–64 μ, flask-shaped with a spherical venter, 16–43.2 × 22.4–51.2 μ, with a distinct dome-shaped columella; venter surmounted by a long neck, 6.4–11.2 × 54.4–200 μ, apex of the neck slightly broader, 8.0–14.4 μ in diameter, closed with a mucilaginous plug. Sporangiospores walled, oblong, 1.4–2.1 × 2.8–4.2 μ, discharged through the neck by the dissolution of the apical plug; during germination usually one germ tube formed.

In soil, Patharia forest in Sagar, Madhya Pradesh State, India.

The peculiar and characteristic shape of the sporangium recalls to mind the sporangia of *Nephrochytrium*, *Catenomyces* and

Rhizidiomyces (see Karling **1944**, Hanson **1945** and Bessey **1950**, Figs. 18 and 22D). In these fungi the sporangia have distinct necks through which zoospores are liberated. It will be interesting to note here that in a species of *Catenomyces* the apex of the neck shows an accumulation of a thick viscid substance (mucilage ?) prior to dehiscence as in the case of the present fungus. This resemblance does not go very far as these three fungi are zoosporic forms and do not have nonmotile endospores as in the present fungus. In addition to this, the sporangium in the present fungus has a distinct and well-formed columella.

On the other hand this mode of sporangial dehiscence with a definite neck does not occur in any of the known Mucorales. This strange resemblance to the chytridiaceous members reminds one of the suggestion of von Jaczewski (1929–30) that the Zygomycetes may have been evolved from the chytridiaceous fungi (see also Bessey **1950**, p. 635 and Fitzpatrick, **1930**, p. 47). In the case of the present mucoraceous fungus this would have involved two steps: (1) the formation of a columella and (2) the formation of nonmotile sporangiospores instead of zoospores.

SUMMARY

A new mucoraceous fungus isolated from a forest soil in India is described in detail.

The fungus is characterized by a stalked flask-shaped columellate sporangium. A large number of sporangiospores are formed and are liberated through the neck by the dissolution of an apical mucilaginous plug.

This fungus is quite unique and does not agree with any of the known genera and hence it is placed in a new genus, *Saksenaea,* the fungus itself being named *Saksenaea vasiformis.*

The affinities of the genus are briefly discussed.

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The writer wishes to express his grateful thanks to Dr. R. K. Saksena for his kind guidance and to Prof. J. N. Couch for kindly perusing the manuscript and making numerous suggestions for

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STUDIES OF SUB-TROPICAL RUSTS. I. PROSPODIUM TRANSFORMANS [1]

FLOYD S. SHUTTLEWORTH [2]

(WITH 9 FIGURES)

Although the Uredinales have been studied cytologically since the publication of Schmitz's (23) paper describing the binucleate condition found in the mycelium and urediospores of *Coleosporium campanulae* (Pers.) Lév., there is much yet to be learned concerning the morphology and nuclear behavior exhibited by this important group of obligate parasites. Especially have the tropical and sub-tropical species been neglected. In the opinion of Jackson (16) and most other modern students of the Uredinales, the short-cycle forms are considered to be the most recently derived species of rusts and thus are the expression of the present day evolutionary trend. Accordingly, there is greater variation among the microcyclic species than among the macrocyclic ones.

In order to determine whether the sub-tropical short-cycle forms exhibit the same degree of variation as do the temperate forms which have been investigated, a study was begun of several sub-tropical microcyclic species, including *Prospodium transformans* (Ell. & Ev.) Cumm., a parasite of *Tecoma stans* (L.) HBK. This rust occurs in South Florida and throughout the West Indies.

[1] This paper is a portion of a thesis submitted to the Faculty of the Graduate School in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Botany, Indiana University, November, 1950.

[2] The author wishes to express his gratitude to Dr. T. R. Alexander, Mr. M. J. Soule, Jr., and other members of the staff, Department of Botany, University of Miami; to Mr. Donald Lawrance, Nassau, Bahamas; to Dr. G. B. Cummins, Arthur Herbarium, Purdue University; and to Dr. H. J. Brodie, Department of Botany, Indiana University.

MATERIALS AND METHODS

Material of *Tecoma stans,* infected with *Prospodium transformans,* was collected at Coral Gables, Florida, and Nassau, New Providence, Bahamas. Collections were made in Florida throughout the year and in the Bahamas during July and December. Leaf, stem, and fruit specimens were obtained.

A number of solutions were used as fixatives, including acetic-acid-alcohol, Bouin's, Randolph's modification of Navashin's fluid (CRAF) (18), FAA (22), and Flemming's fluid, weaker solution (18). The last two fixatives proved to be the most satisfactory for this study. After dehydration with tertiary butyl alcohol (18), the material was imbedded in paraffin and was sectioned on the rotary microtome at 5, 7, 10, and 15 μ. Fresh material was also cut on the freezing microtome at 10 and 15 μ, using a razor blade holder with a single edge blade instead of the special knife ordinarily used with the freezing microtome. For the large galls formed by *P. transformans,* the freezing microtome method was more satisfactory than the paraffin method.

Of the various staining techniques employed, Tuan's (26) iron-alum hematoxylin-picric acid method, Harris' hematoxylin (18), Flemming's triple stain (18), and the Feulgen reaction (10) gave the most satisfactory results.

For the study of germination, the spores were placed in a drop of water on a slide which was inverted over two glass rods in a petri dish containing filter paper soaked with distilled water. Better permanent mounts were obtained by first placing a thin film of 1% water agar on the slide (12) and adding a drop of spore suspension after the agar had dried. In both methods, after germination had taken place, the slides were air-dried, fixed with CRAF, FAA, or osmic acid fumes and then stained with aceto-carmin for immediate study or with one of the two hematoxylin methods or with the Feulgen reaction for permanent mounts.

For the inoculation studies, small plants of *Tecoma stans,* potted in good soil, were sprayed with a fine mist of distilled water, placed in an airtight glass container which had also been sprayed with distilled water, and inoculated with teliospores germinated in a petri dish of distilled water. Control plants were treated in the same manner except that the spore inoculation was omitted.

RESULTS AND DISCUSSIONS

Prospodium transformans parasitizes two species of *Tecoma* (*Stenolobium*) throughout its geographic range. This rust is distributed from southern Florida and the Bahamas to Mexico, Cuba, and Guatemala (9). A microcyclic species which forms only pycnia and telia, it is considered to be correlated with the macrocyclic *P. appendiculatum* (Wint.) Arth. (9). In Florida, *P. transformans* and *P. appendiculatum* usually occur on the same host plant, the telia of the former and the uredia of the latter species often on the same leaf. However, during the three years of this investigation, the author did not observe the pycnial-aecial gall-forming stage of the macrocyclic species in Florida, although the uredial stage was abundant and the telial stage not too uncommon.

Pycnia of *P. transformans* are amphigenous, caulicolous, or fructiculous, yellow-brown in color, subcuticular in position, and conical in shape. They are often overlooked as they are soon replaced by the telia. Pycniospores are spherical in shape and uninucleate.

The telia may cause slight to severe hypertrophy on leaves, or even extensive galls (FIG. 1) on petioles, stems, and fruits. The galls reach a diameter of over 3 cm. and are more or less spherical or sometimes longer than wide. Internally the galls are composed of greatly enlarged parenchymatous cells, which have increased not only in size but in number as well. The vascular tissue is small and scattered throughout the gall. Galls can occur on *Tecoma* anywhere from the base of the stem to just below the terminal bud. Because these galls are composed of such thin-walled cells, the stems which are so attacked are prone to break off during hurricanes and severe wind storms. This may be one of the principal reasons why *Tecoma stans* seldom attains tree size in Florida, remaining usually a shrubby plant with many main stems arising from the base. The interior of the gall is packed with intercellular hyphae which have prominent haustoria, often coiled as described by Evans (13) and Rice (21) for other rust species.

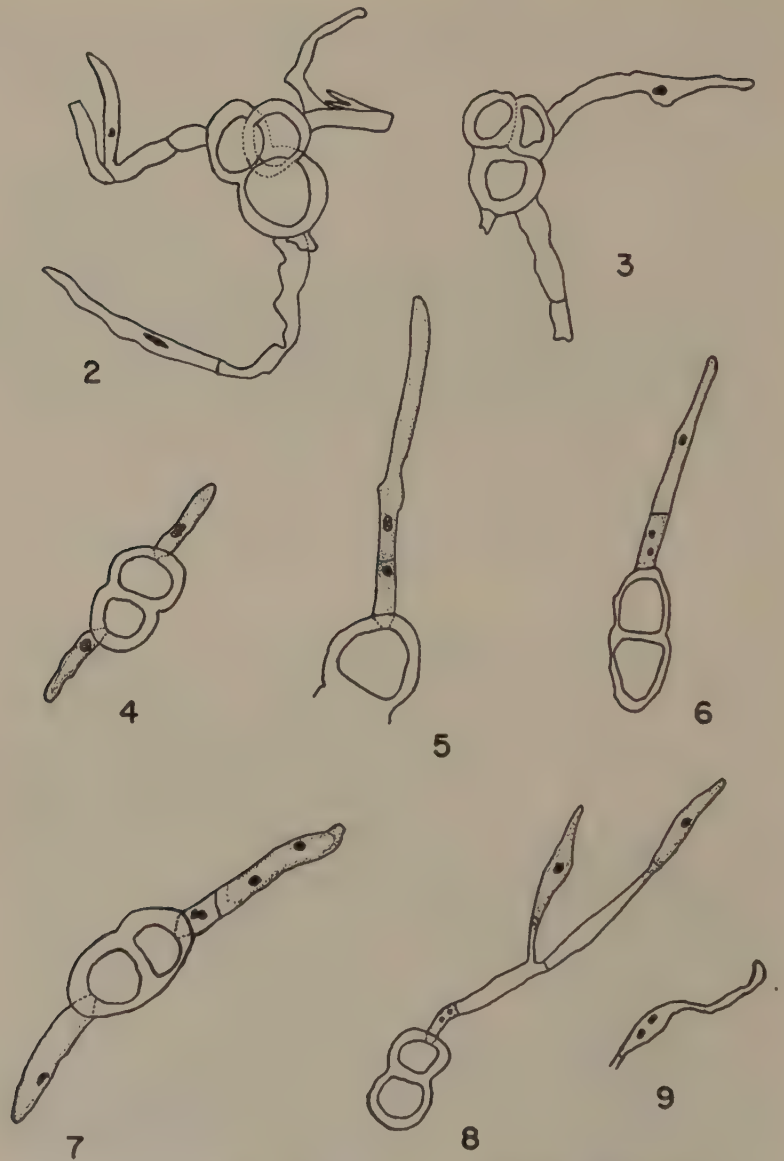
Teliospores are borne on the surface of the hypertrophied areas or on the entire surface of the galls. Sometimes the teliospores

are borne internally within the gall itself. Internal sori have been reported for a number of species (1, 29), and Colley (8) has termed them "teratologic phenomena of no special morphologic significance."

The teliospores are leptosporic; that is, they germinate immediately upon maturation if the environmental conditions are satisfactory. A high humidity is necessary for germination. However, when all other conditions are satisfactory, temperature is the limiting factor. Suspensions of spores kept at 10° C did not germinate, and only one to two per cent germinated at summer room temperature (27°–31° C). About ninety per cent germination was obtained at 18°–21° C. Exposure to this temperature range for only one or two hours and then removal to room temperature did not decrease the amount of germination. Cochrane (7) found that brief exposure to optimum temperatures materially increased the germination of urediospores of a species of rust held at either high or low temperatures. Suspensions of teliospores of *P. transformans* kept for 24 hours at either 10° C or at room temperature germinated abundantly when transferred to an airconditioned room with a temperature range of 18°–21° C. This temperature range appears to be near the optimum for this species. This temperature is slightly lower than that found for teliospores of *Endophyllum heliotropii* Thirum. and Navasimhan (25) and corresponds more nearly to that found as optimum for the urediospores of a number of species in northern areas (7, 14, 27).

The teliospores of *P. transformans* are normally two-celled and break off from the pedicel near the base of the spore. The germ pore is apical in the upper cell and is next to the pedicel in the lower cell. A few three-celled teliospores were observed. In such spores the lower cell was normal, the upper two cells separated by a vertical septum. The upper two cells were each near normal size, with germ pores lateral, although actually apical in relation to the vertical cross wall. The multiplication of cross walls is, according to Arthur *et al.* (1), of common occurrence in the Pucciniaceae, but such variation in the structure of the spore, due to number and position of septa, may be considered as abnormal development and of no special significance.

FIG. 1. Galls formed on *Tecoma stans* as result of infection by *Prospodium transformans*. Upper photograph, main stem in which galls have nearly girdled the stem. Lower photograph, galls on young twig and on leaf petiole. These galls are covered with mature teliospores.



FIGS. 2–9. Germinating teliospores of *Prospodium transformans*. 2. A three-celled teliospore showing all three cells germinating. Germination abnormal. 3. A three-celled teliospore with two cells germinating. Portion of one tube broken off. 4. Normal two-celled teliospore, both cells germinating, each showing the fusion nucleus which has migrated out into

Although there have been reports of teliospores having supernumerary cells, there have not been, apparently, any reports concerning their germination. FIG. 2 shows one such spore in which all three cells have germinated, the upper left teliospore cell having a definite two-celled basidium, with one somewhat abnormal basidiospore and the other cell of the basidium broken off. FIG. 3 illustrates one three-celled spore in which two cells have produced germ tubes.. The end of one tube was broken off in the preparation of the slide.

NUCLEAR BEHAVIOR

The young teliospores of *Prospodium transformans* are binucleate and arise from binucleate cells at the base of the sorus. The mature teliospore is uninucleate indicating that a fusion has taken place. This is the only true diploid uninucleate cell in the life cycle of this rust. At what place and in what manner the binucleate condition arises has not been determined. The pycnia and the pycniospores are uninucleate, and most intracellular hyphae also appear to be uninucleate. No evidence of fusion cells or nuclear migrations, as reported (2, 6, 28) for other species of rust, was found. Olive (19) reported fusion cells for *Prospodium* (formerly *Puccinia*) *transformans,* but, according to Jackson (16), the species with which Olive was working was *P. elegans* (Schroet.) Cumm. and not *P. transformans.* The binuceate condition in *Puccinia malvacearum* Bert. was reported by Ashworth (2) and by Brown (4) as arising from a nuclear division without cross wall formation, and thus the two nuclei are sister nuclei and not a true dicaryon. This latter species is, according to Brown (4), homothallic. Although attempts were made to obtain single basidiospore infections with *Prospodium transformans,* none were obtained, and, therefore, it is unknown whether this species is

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the tube. 5. Germ tube with one nucleus in stalk cell and one nucleus in basidial initial. 6. Germ tube in which nucleus of stalk cell has divided but nucleus in basidial initial has not. 7. Teliospore with a 4-nucleate germ tube and a 1-nucleate germ tube. 8. Mature two-celled basidium. Two nuclei in stalk cell starting to degenerate. Basidiospores uninucleate. 9. Binucleate basidiospore which has started to germinate while attached. Nuclear division precocious. Cytoplasmic contents not shown.

homothallic or heterothallic. The presence of well-developed pycnia suggests that this species is probably heterothallic.

Upon germination, the teliospore produces a two-cell basidium, supported by a very short basidial stalk cell. Each cell of the basidium proper produces a single basidiospore, uninucleate at first, but often becoming binucleate by mitotic division as the spore begins to germinate *in situ*. Two-celled basidia are known for several species of rust, but in most cases the nuclear behavior in such species is unknown. The species in which the nuclear behavior has been studied are discussed by Jackson (17).

The first meiotic division of the fusion nucleus may take place within the teliospore but more commonly occurs within the germ tube, usually near its point of emergence from the spore. FIG. 4 illustrates the fusion nucleus which has migrated into the germ tube but has not yet divided. FIG. 5 shows the result of the first meiotic division in which the two nuclei are separated by a septum before undergoing the second meiotic division. In some cases the second division appears to occur in both nuclei at approximately the same time: in other cases (FIG. 6) the nucleus in the stalk cell may divide before the one in the basidial initial divides. In either case, two of the resulting four nuclei remain in the stalk cell, where they eventually degenerate, leaving the stalk cell empty of all protoplasmic material.

The second nucleus of the first meiotic division divides again in the basidial initial either before (FIG. 7) or after the sterigmatal branch begins to protrude. This side protuberance ordinarily remains short and acts as a sterigma rather than a true branch. The end portion of it swells into a basidiospore and is densely filled with cytoplasm. The main portion of the germ tube elongates and the upper end constricts and then swells to form the second basidiospore. A cell wall is laid down just above the area where the side protuberance originated. The two nuclei, one in each cell of the basidium, migrate into their respective basidiospores, the one going to the terminal basidiospore often being much drawn out as it passes through the constriction of the sterigma into the spore. Vacuolization at the base of the spores occurs, and a wall is laid down cutting off each spore (FIG. 8).

The basidiospore, while still attached to the basidium, may begin to germinate, sending out a long, narrow, and often curved tube, densely filled with cytoplasm. In such a spore, there may be two nuclei (FIG. 9) which have arisen by precocious division without the formation of a cross wall.

Jackson (17) has summarized the six types of variation found in microcyclic rusts. The majority of these are known for Endo-forms because in very few Micro-forms has the nuclear detail of germination been studied. The six types of variations as given by Jackson are as follows:

"1. The mycelium is at first uninucleate, becoming binucleate in connection with the development of the sorus plexus. A fusion of the two nuclei occurs in the young teliospore or aeciospore. Meiosis follows, resulting in four nuclei and a four celled basidium and uninucleate basidiospores. . . .

2. The mycelium is uninucleate, becoming binucleate. In the young spore (morphologically the aeciospore) no fusion occurs. The two nuclei pass into the developing basidium and each divides once. Septa are laid down between each of the nuclei and the mature basidium is four celled. Four uninucleate basidiospores develop. . . .

3. The mycelium is binucleate throughout. The nuclei fuse in the young teliospore. In the developing basidium a septum is laid down following the first meiotic division. No septum is formed after the second division. The mature basidium is two celled, each basidiospore receiving two nuclei, the product of the second meiotic division. . . .

4. The mycelium is at first uninucleate, becoming binucleate. The young teliospore is binucleate but no fusion occurs. One of the two nuclei degenerates. The remaining nucleus passes into the promycelium and is isolated by a cross wall. It then divides once. A cross wall is laid down between the daughter nuclei. The mature basidium is two celled but a basidiospore is formed only from the upper cell, the nucleus in the lower cell degenerating. . . .

5. The mycelium throughout is uninucleate as well as the young teliospore. In the basidium the single nucleus divides once and a septum is laid down between the resulting nuclei. The mature basidium is two celled, the basidiospores uninucleate. . . .

6. The mycelium is uninucleate throughout, the nucleus of the young teliospore passes into the basidium and divides twice. The mature basidium is four celled and the basidiospores uninucleate. . . ."

To these six variations, Thirumalachar and Narasimhan (25) have added a seventh type found in *Endophyllum heliotropii*. In this rust the mycelium is binucleate throughout. The teliospores are binucleate and the nuclei do not fuse, but move out into the basidium where they do not divide. They are separated by a septum, forming a two-celled basidium. The nuclei move into the basidiospores where they divide, forming binucleate basidiospores which give rise to a binucleate mycelium.

To these seven variations must now be added an eighth variation, namely that found in *Prospodium transformans*, described in this paper. The mycelium is uninucleate, becoming binucleate. The young teliospores are binucleate, but fusion occurs in the mature spore. Meiosis results in four nuclei, two of which degenerate within the basidial stalk cell. A two-celled basidium is cut off, and each basidiospore is uninuculeate.

From this summary it is evident that the microcyclic species of rusts exhibit great variation, and further studies, especially of the tropical and sub-tropical forms, will probably show that still other variations occur.

CELLULAR INCLUSIONS

In the rust infected tissue of the leaves of *Tecoma stans*, the abundance and size of the cellular inclusions were so striking that certain cytochemical tests were made to determine their nature. The physical nature of these inclusions and the manner in which they coalesce suggest the coacervates described by Bungenberg de jong (5), Dobry (11), Humphrey and Dufrenoy (15), Reed (20), and others, although they did not give the color reactions which the above workers obtained with the molybdenum reagent. The inclusions were stained by all of the basic stains applied, including the basic fuchsin of the Feulgen reaction. Nuclei and haustoria could often be seen in the same cells with the inclusions. A detailed account of this phase of the present investigation is to be published elsewhere.

INOCULATION EXPERIMENTS

Young plants of *Tecoma stans* apparently free of rust infection were obtained from northern Florida. They were inoculated in

the manner described earlier in this paper, and control checks were given the same treatment except that the spore suspension was omitted. Pycnia and telia appeared in 18 to 21 days in most cases, but one plant produced no visible signs of infection before 35 days. Pieces of infected and control leaves were killed, fixed, and paraffin sections made. Galls did not appear on the stems or petioles during the five months of observation, although the leaves were hypertrophied where the pycnia and telia formed. The control plants exhibited no signs of infection at the end of the observation period.

SUMMARY

1. A sub-tropical microcyclic species of rust—*Prospodium transformans* which parasitizes *Tecoma stans*—was investigated. Material was collected in South Florida and in the Bahamas.

2. A variety of fixing solutions and staining methods was used. Both paraffin sections and freezing microtome sections were made.

3. *P. transformans* causes hypertrophied areas on leaves, and large galls on stems, petioles, and fruits. The host tissue in such areas is parenchymatized and the vascular bundles are modified and scattered within the galls.

4. These galls are packed with intercellular hyphae which have large haustoria within the host cells.

5. Internal sori are often formed within the galls.

6. *P. transformans* produces both pycnia and telia. The former are uninucleate, the latter binucleate at the base of the sorus and in the young teliospores.

7. Fusion takes place within the mature teliospore. Germination of the spore results in a two-celled basidium. The fusion nucleus undergoes two meiotic divisions, and two of the four resulting nuclei remain in the basidial stalk cell where they degenerate.

8. The basidiospores are uninucleate when formed. They begin to germinate *in situ* and often become binucleate by a precocious division of the nucleus.

9. The optimum temperature range for the germination of the teliospores of *P. transformans* is apparently 18°–21° C.

10. The type of nuclear cycle displayed by *P. transformans* differs from all of the six variations described by Jackson and also from the seventh type of variation recently reported by Thirumalachar and Narasimhan.

11. Within the leaves of *Tecoma stans* infected with *P. transformans* are formed cellular inclusions, interpreted to be coacervates of undetermined chemical composition.

12. Inoculations usually produce pycnia and telia after 18 to 21 days. Sometimes longer periods are necessary for visible signs to appear.

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A UNISEXUAL MALE CULTURE OF CHALARA QUERCINA [1]

H. L. Barnett

(with 3 figures)

Most of the workers who have studied a number of isolates of *Chalara quercina* Henry in culture have noted considerable variation in the cultural characteristics of the isolates. Henry (3) was the first to note these differences, which are mainly in appearance and growth rate of mycelium and in abundance of conidia. Bretz (1) has shown that the fungus is heterothallic and capable of producing perithecia and ascospores in culture. Hepting *et al.* (5) have recently reported that single ascospore cultures fall into two groups designated as A and B. Each single spore culture is typically bisexual (hermaphroditic), capable of acting both as male and as female, self-sterile and cross-fertile. The conidia act as spermatia. In this respect *C. quercina* is apparently quite similar to *Hypomyces solani* f. *cucurbitae* as reported by Hansen and Snyder (2).

The present paper reports the results of a portion of a broader study that deals with the crossing of isolates obtained from different states in the oak wilt region. More specifically, this paper reports an isolate of *C. quercina* which has the ability to act as male but which apparently has lost the ability to act as a female.

MATERIALS AND METHODS

All cultures used in the present study originated from single spores of isolates from oak wilt trees. All isolates numbered in the 600 series are from West Virginia. Culture *805* is a transfer from a Minnesota isolate No. *R39* obtained through the courtesy of Dr. D. W. French. It was isolated August, 1950. Cultures *808* and

[1] Published with the approval of the director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 453.

810 are from Iowa, obtained through the courtesy of Dr. W. H. Bragonier.

Considerable time and effort were spent in the selection and improvement of a semisynthetic medium which could be duplicated easily and which would be favorable for the production of perithecia. The medium finally selected had the following composition: maltose 5 g.; Difco Casamino Acids (technical grade) 1 g.; KH_2PO_4 1 g.; $MgSO_4 \cdot 7H_2O$ 0.5 g.; Zn and Fe 0.2 mg.; Mn 0.1 mg. (as sulfates); biotin 5 μg.; distilled water 1 liter; agar 20 g. The pH of the medium should be adjusted to approximately 6.0 before autoclaving at 15 pounds pressure for 15 minutes. If no pure biotin is available the partial deficiency of some isolates may be overcome by adding 0.5 g. yeast extract to the medium.

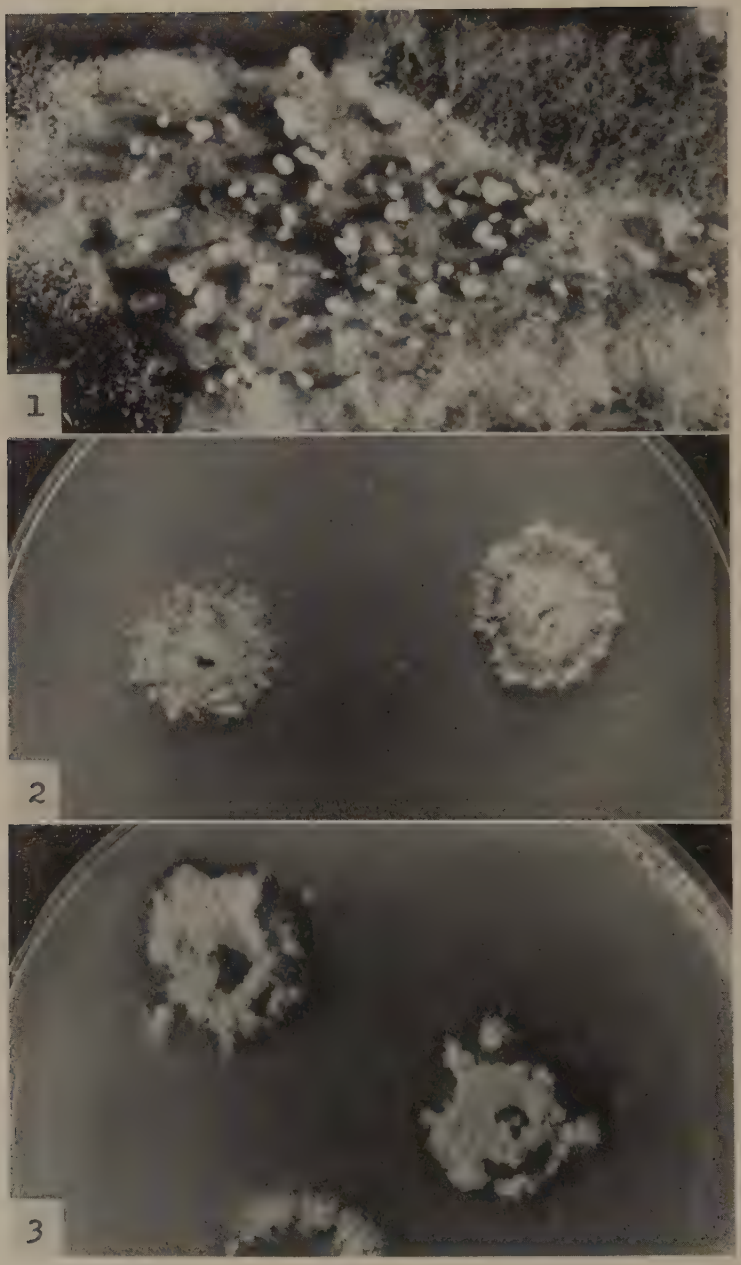
Petri plates of the above medium were inoculated at three points with pieces of mycelium which were allowed to grow for six days at 25° C. At this time the cultures were spermatized by placing loopfuls of conidial suspension on the mycelium or by spraying the mycelium with a spore suspension using an atomizer. Three days later the cultures were examined by means of a stereoscopic wide field microscope. Cultures having no perithecia were examined again two days later. The presence of perithecia producing ascospores indicated a fertile cross. All crosses were repeated once or twice.

EXPERIMENTAL RESULTS

Thirty isolates, each from a different tree in West Virginia, were crossed with four cultures used as standard in order to determine the compatibility group and crossing ability of each isolate. Fourteen were of group B and 16 belonged to group A.[2] All were bisexual, capable of acting both as male and as female, self-sterile and cross-fertile. These results verify the findings of Hepting *et al.* (4). The production of perithecia on maltose-Casamino Acids medium is shown in FIG. 1.

Five Minnesota isolates were then used as female cultures in crosses with four West Virginia isolates as male. The results are

[2] For the sake of uniformity A and B are applied to the same compatibility groups first designated A and B by Hepting *et al.* (4).



FIGS. 1–3.

given in TABLE I. The presence of fertile perithecia is indicated by P, their absence by O.

TABLE I

RESULTS OF CROSSING MINNESOTA ISOLATES USED AS FEMALES WITH WEST VIRGINIA ISOLATES

W. Va. \ Minn.	A *801*	B *802*	B *803*	B *804*	A *805*
B *611*	O	P	P	P	O
B *613*	O	P	P	P	O
A *621*	P	O	O	O	O
A *638*	P	O	O	O	O

Isolate *805* was unique in that it failed to act as female in crosses with 4 West Virginia isolates of either A or B group. Reciprocal crosses showed that all of the Minnesota cultures were capable of acting as males. The crosses between *805* used as male and West Virginia B cultures as female produced perithecia and ascospores. Therefore, *805* belongs to the A group.

The crosses were then expanded to include other cultures from West Virginia, Minnesota and Iowa as male and two single spore isolates of *805* as female. The results are given in TABLE II.

TABLE II

RESULTS OF CROSSING TWO SINGLE-SPORE CULTURES OF *805* AS FEMALE WITH 9 OTHER CULTURES USED AS MALE

	B *801*	A *808*	B *810*	B *611*	A *620*	B *622*	A *624*	B *625*	A *638*
A *805-1*	O	O	O	O	O	O	O	O	O
A *805-2*	O	O	O	O	O	O	O	O	O

These results strongly indicate that isolate *805* is a unisexual male, incapable of acting as a female. Further evidence was obtained from 4 additional single spore isolates of Minnesota *R39* (the source of *805*) made by D. W. French. None of these produced perithecia when spermatized with conidia of test West

FIG. 1. Perithecia and light masses of ascospores produced in culture, × 10.

FIG. 2. Culture *805*, 6 days old. Note the light mycelium with small aerial tufts.

FIG. 3. Culture *633*, 6 days old. Note the dark smooth mycelium.

Virginia isolates. The macroscopic appearance of isolate *805* distinguishes it from most other isolates. The mycelium is light on the above medium and it forms numerous small aerial tufts (FIG. 2). In contrast, the majority of the isolates produce dark mycelium which may be uneven in its growth but does not produce the small tufts. It must be emphasized that the color of the mycelium varies with the medium used. Light and dark mycelia as described in this paper were compared on the maltose-Casamino Acids medium. Apparently they are the same forms which Henry (3) described as "white" and "normal."

If isolate *805* is genetically unable to act as a female, a cross with a bisexual isolate should give F_1 single-ascospore isolate, half of which would be bisexual and half unisexual males. To test this hypothesis, isolate *633* (bisexual, dark mycelium, FIG. 3) was selected as a typical culture to use as female in cross with *805*.

From this cross 23 single ascospore cultures were obtained. It was soon apparent that some of the cultures were like *633* in appearance while others were like *805*. These F_1 isolates were then used as females in crosses with 4 West Virginia isolates, two of which were A group and two of the B group. The results are presented in TABLE III. It was necessary to make reciprocal crosses to determine the compatibility group of some of the isolates and to determine that they could act as males.

TABLE III shows that the 23 single ascospore cultures of cross *633*, dark B ♀ × *805*, light A ♂ segregated into four groups as follows: 14 dark B ♀, 2 dark A ♀, 6 light B ♂, and 3 light A ♂. These results show that the character unisexual male is inherited genitically and that isolate *805* is genitically unable to act as female. The factor for compatibility is not linked with sex or with color of mycelium. In the particular cross above dark mycelium segregated with the bisexual character and light mycelium with the unisexual male character.

The above cross was repeated and of the 57 single-ascospore cultures obtained 27 were dark and 30 were light. In crosses using these F_1 isolates as females and *613* and *638* as males, 24 of the dark isolates produced perithecia. Three dark cultures and all of the light cultures failed to act as females. This is further evi-

TABLE III

RESULTS OF CROSSING SINGLE ASCOSPORE CULTURES OF THE CROSS (*633* ♀ × *805* ♂) AS ♀ WITH FOUR WEST VIRGINIA CULTURES AS ♂

♀ \ ♂	B *611*	B *613*	A *620*	A *638*	Compatibility group	Color	Sex
7	O	O	P	P	B	dark	bisexual
8	O	O	P	P	B	dark	bisexual
9	O	O	P	P	B	dark	bisexual
11	O	O	P	P	B	dark	bisexual
14	O	O	P	P	B	dark	bisexual
15	O	O	P	P	B	dark	bisexual
16	O	O	P	P	B	dark	bisexual
17	O	O	P	P	B	dark	bisexual
19	O	O	P	P	B	dark	bisexual
20	O	O	P	P	B	dark	bisexual
21	O	O	P	P	B	dark	bisexual
23	O	O	P	P	B	dark	bisexual
5	P	P	O	O	A	dark	bisexual
18	P	P	O	O	A	dark	bisexual
2	O	O	O	O	B*	light	male
4	O	O	O	O	B	light	male
6	O	O	O	O	B	light	male
10	O	O	O	O	B	light	male
22	O	O	O	O	B	light	male
24	O	O	O	O	B	light	male
12	O	O	O	O	A	light	male
13	O	O	O	O	A	light	male
15	O	O	O	O	A	light	male

* Compatibility of male cultures was determined by reciprocal crosses.

dence that there may be genetic linkage between color and sex. Loss of femaleness is apparently usually accompanied by loss of dark color. However, the inheritance of color is not fully understood and may be complex.

Since it is possible that the medium may affect the fertility of a cross, several of the unisexual male F_1 isolates were tested in duplicate crosses on chestnut meal agar, a medium used by some workers for the production of perithecia. The results on this medium did not differ from those on the standard maltose-Casamino Acids agar.

DISCUSSION

While the finding of unisexual male cultures of *C. quercina* is not surprising, it lends evidence to the idea held by many that this

fungus is quite variable and changeable. It is not known whether these light colored unisexual male mutants occur in nature, but there is evidence that this degenerate change occurs rather frequently in culture.

Several isolates, some of which have been in culture for less than one year, have failed to act as female in a limited number of crosses. Although genetic proof is available at present for only one isolate, it seems probable that a number of these isolates are true unisexual males. Their frequency might explain, at least in part, the difficulty some workers have experienced in obtaining perithecia in culture.

The medium and the method of growing and spermatizing the cultures described above were developed specifically for the production of perithecia. The method is similar to that used by Hepting *et al.* (**5**). They are now believed to be sufficiently dependable that only two or three repetitions are necessary to establish that a certain cross is sterile.

The similarity between *C. quercina* and *Hypomyces solani* f. *cucurbitae* is again apparent in the existence of unisexual males, reported for the latter fungus by Hansen and Snyder (**2**). When cytogenetic studies of *C. quercina* are made they should be compared with the findings of Hirsch (**6**) in *H. solani* f. *cucurbitae* to determine whether the loss of femaleness can be explained on the same genetic basis in both fungi.

SUMMARY

In crosses between isolates from Minnesota and West Virginia, culture *805* failed to act as female in repeated trials. When this culture having light mycelium was crossed with a bisexual culture with dark mycelium, the 23 F_1 single ascospore cultures segregated into light male and dark bisexuals. The compatibility factors separated independently of sex or color of mycelium. Other isolates have shown this apparent inability to act as female. It is not yet known whether unisexual male mutants occur in nature.

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ADDITIONAL OBSERVATIONS ON THE CHROMOSOMAL STRUCTURE OF THE VEGETATIVE NUCLEUS OF BLASTOMYCES DERMATITIDIS [1]

EDWARD D. DELAMATER

(WITH 11 FIGURES)

In a previous study of the nuclear cytology of *Blastomyces dermatitidis* (1) by means of new methods (2), the author described the nuclei and their mitotic activity in relative detail. The accompanying plate presents additional observations on the chromosomes of this organism.

The methods used are the same as those previously described. The cells of *B. dermatitidis* are multinucleate. FIGS. 1–5 show five successive focal planes through the same cell, and demonstrate two nuclei lying at either end of a cytoplasmic band which bisects the cell. The nuclei lie within the cytoplasm and adjacent to the vacuoles, but are distinct from the vacuoles. The chromosomes within the upper nucleus, which is in interphase, are especially well seen as long, delicate, beaded threads.

FIGS. 6–8 represent three focal planes through the same cell, showing a cluster of interphase nuclei, again within which the chromosomes appear as tangled, beaded threads. The dark granules in the nuclei in FIG. 6 represent chromosomes in cross section. In FIG. 9 in a dying cell are seen thread-like beaded chromosomes. The nuclear membrane has been disrupted. In FIGS. 10 and 11 are shown two optical levels through a prophase nucleus in which four contracting and beaded chromosomes were made out in the original preparation.

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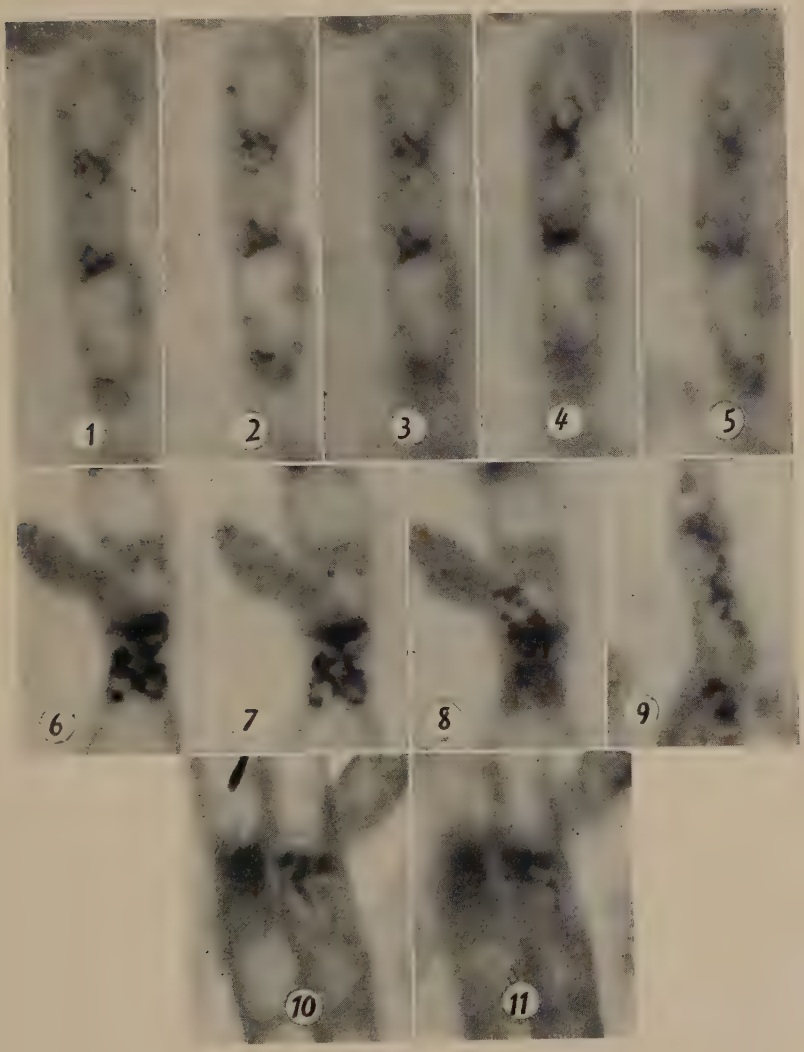

Figs. 1–11.

CONCLUSION

The observations presented add to those previously documented concerning the nature of the chromosomes and their number in the multinucleate cells of *B. dermatitidis*. These observations are presented because it is felt that additional evidence concerning chro-

mosomal structure and activity in the bacteria and fungi will be useful.

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NOTES ON SOME MYCOLOGICAL METHODS

M. J. Thirumalachar and M. J. Narasimhan

(with 1 figure)

During the course of mycological and plant pathological studies the authors have been employing for the last several years some research techniques which have been found to be very useful and warrant recording.

1. A water mounting medium for making permanent microscopic preparations

The need for a medium which would render the slides permanent by direct mounting from water or lactophenol has been keenly felt by all students of fungi. The benefits of preserving permanently hundreds of interesting observations made under the microscope have been lost in most cases because the mounts are temporary and can be preserved only for a few weeks or months. Various mounting methods are employed which make the microscopic preparations fairly permanent, but each of them has certain disadvantages of its own. Glycerine jelly, for instance, has been widely used for mounting by students of fungi. While these mounts are fairly permanent, the material becomes opaque and does not have the clarity of objects mounted either in lactophenol or xylol-canada balsam. The double cover-glass method of mounting devised by Diehl (1929) and further modified by Chupp (1940) is really advantageous in mounting filamentous algae and fungi in the absence of any other mounting medium of better quality. Apart from the expensiveness of using two cover-glasses for sandwiching the material, the retention of even a little trace of water in the medium after heating makes the mounts milky-white and opaque due to admixture with xylol used along with canada balsam. Secondly, having made a chance observation of an in-

teresting stage of a fungus under the microscope, it is difficult to transfer the material *en bloc* without mutilation to another cover-glass for making the permanent mount according to the double cover-glass method.

The use of lactophenol as a medium for mounting and staining the materials of fungi has been widely employed. Dade and Waller (**1949**) used a sealing wax mixture consisting of equal parts of refined beeswax and gum dammar for sealing the margins of the cover-glasses of lactophenol mounts and thus rendering them semi-permanent. Thirumalachar and Pavgi (**1950**) found that the use of canada balsam of thick consistency as an additional coating over the beeswax-gum dammar sealing medium made the mounts more permanent even under the conditions prevailing in the tropics. However, in all these cases the purpose in sealing the margin of the cover-glasses is to create an effective barrier against the outflow of the lactophenol which often happens even with the slightest break of contact between the slide and the sealing medium. In the present studies the authors have been employing a water mounting medium which renders the mount permanent and hard, like canada balsam, and at the same time retains the clarity of the objects. The medium has the following composition:

Gum arabic	20 gm
Chloral hydrate	10 gm
Dextrose	10 gm
Glycerine	3 ml
Distilled water	30 ml

Powdered gum arabic is first placed in distilled water until it becomes a uniform gel. Chloral hydrate and dextrose are added to this and slightly warmed until a uniform viscid solution is formed. Glycerine is added to this solution and well mixed by stirring.

For making permanent mounts the material on the slide may be sealed with a cover glass using the water mounting medium. Where staining of the material is required, the objects are stained with cotton blue or acid fuchsin in lactophenol and slightly warmed until proper differentiation is obtained. After this, any excess of lactophenol is drained off and the material mounted in the water

mounting medium described above. The slides are then placed in an incubator or on hot plate at 40 to 45° C for 48 hours. When the medium has dried up and become sufficiently hard, the margin of the cover glass is streaked with a solution of medium consistency of canada balsam in chloroform and the slides are left on the hot plate for a few hours. This additional coating of canada balsam on the dried water mounting medium along the margin of the cover glass, prevents the possible re-absorption of moisture and consequent softening which sometimes happens under excessively humid weather conditions.

Depositing of the type specimens in important herbaria of species of fungi described as new to science is considered by mycologists as important. While these serve the purpose of being available for future comparative studies, in many fungi, such as species of *Sclerospora,* the herbarium material is without value for studying the conidial stages. Even so, in genera of fungi such as *Phyllosticta, Cercospora* and several others, the spores disappear after a few years of storing in the herbarium. It would be in the interest of mycological science to insist on the deposit of at least three slides made from the type material to be included in the same packet with the type specimen. This would enable the person making comparative studies at a later date to obtain the picture as seen by the author of the species. Considering the ease with which permanent mounts could be made by using the water mounting medium described above, we feel confident that the depositing of the slides with the herbarium material of the type specimen would not be a difficult task.

2. AN IMPROVED METHOD FOR GERMINATING AND STAINING THE SPORES OF FUNGI

For germinating and staining spores of various fungi such as the teliospores of rusts, chlamydospores of smuts etc. one of us (Thirumalachar, **1940**) described a procedure for fixing the spores to the slides. The spore material to be germinated was fixed to the slide by alternate wetting and drying of the spore mounts. The spore material thus fixed to the slide was inverted over a water surface supported by racks, and wet paper toweling was

placed on the back of the slide to help condense films of water on the inner side. Use of small card-board boxes which were rendered water-proof by dipping in molten paraffin was recommended by Thirumalachar and Pavgi (1950) as improvised moist chambers for incubating the slides. Further experience with this germination technique showed that when the germinated spores were carried through staining solutions, grades of alcohol etc. in the process of staining, several of the spores got dislodged from the slides and floated away, resulting in the loss of valuable stages in the germination. The writers have been investigating for the last several years the possibility of finding an adhesive which could be used to fix the spores to the slides prior to placing them for germination. Such an adhesive should meet the following requirements: (a) the adhesive should not inhibit or interfere with the spore germination; (b) it should not be dissolved in water and thus loosen the spore mass whch has been fixed to the slide; (c) it should not incite the growth of bacteria and other contaminants during incubation in moist chamber; (d) it should not show avidity for the stains used for staining the germinating spores. A large number of gums from different plant sources: resins, gum dammars, albumen, etc. were tried without success. Mucilages of various plants were also tried. Of the several that were tried, the mucilage from the stems and flowering stalks of *Tradescantia* sp. (*T. virginiana* L.?), a plant grown in almost every garden for its bright colored foliage, was found most useful. The writers have used this for fixing the spores to the slides with excellent results and feel confident that this germination technique would afford a means to get at critical cytological stages of germinating spores which hitherto has been found difficult on account of lack of a proper method of affixing the germinating spores to the slides for staining. The outline of the procedure for mounting, germinating and staining of the spores is as follows:

The spore material to be germinated is mounted in a droplet of water on a slide. Flowering stalks or young shoots of *Tradescantia* are freshly cut, and by pressing the cut end with the fingers, a tiny drop of the viscid mucilage is squeezed out which is then mixed with the spore material on the slide. If due to some

stage of maturity of the *Tradescantia* plant the mucilage is watery or of thin consistency, such shoots should not be used. With the aid of a needle or scalpel a uniform smear is made which is then air dried. The large number of raphides which are present in the mucilage disappear completely during the process of fixing, mordanting and staining. By alternately moistening and drying the slides, the material gets firmly fixed to the slides. Steeping the slides for a period of 10 to 20 minutes in a solution of potassium permanganate (0.01% or two tiny crystals for a glass of water) has been found to reduce contamination and stimulate spore germination in most of the cases studied. The slide, with the spore material thus fixed, is inverted over a water surface supported on racks and with wet paper toweling on the back side to help in condensing films of moisture by the methods already described (Thirumalachar, **1940**; Thirumalachar & Dickson, **1947**).

When sufficient numbers of spores have germinated, as determined by examination under the microscope, the material is fixed by the following procedure: excess of water surrounding the spore mount is wiped off and a small drop of Allen's modification of Bouin's fluid (with 1% urea) is added and spread over the spore mount which is then allowed to air dry. Immediately after the fixing solution dries, the slides are placed for 5 minutes in water and then transferred to Allen's modified Bouin's fluid in a coplin jar for 2 to 6 hours, depending upon the nature of the material. The slides after fixation are washed, stained, dehydrated in grades of alcohol and mounted in xylol-canada balsam by following the usual procedure adopted for paraffin sections.

The drying up of the slide after the addition of a droplet of Allen's modified Bouin's solution does not cause any shrinkage or distortion of the contents of the delicate germ tube. Actually, on the addition of the fixing fluid (containing picric acid and acetic acid), the mucilage swells, and on drying it prevents shrinkage of the delicate germ tubes. Immediate transfer of the slides to water after the few minutes drying completely restores to normal any possible distortion that might have taken place. The drying up of the slide has a special advantage in cases such as teliospore germinations. In these, usually, the promycelia bearing the sporidia

grow away from the water surface and only the bodies of the teliospores are fixed to the slide, while the promycelium and sporidia remain free-floating. By this drying process even these get fixed to the slide and there is no loss of sporidia etc. from the spore mount.

3. A METHOD FOR PRESERVING AND TRANSPORTING PLANT MATERIALS IN FRESH CONDITIONS

The need for getting diseased plant material in a fresh condition is very essential when isolation and cultural studies of the disease-inciting organism are planned. In many cases the period between

FIG. 1. Photograph of a specimen packet made from the banana leaf sheath, opened to show the specimens and the method of folding of the banana leaf sheath.

the time of collection of the diseased material and the time of microscopic examination may be more than a week (as for instance in the case of materials collected during mycological forays). If the material could be kept in a fresh state for the period it would always permit a more critical study.

Several methods employed at present are as follows: wrapping the material in water-proof paper, celluloid cases, packing in moss

and peat etc. These help to retain the moisture and viability of the spores but do not preserve the fresh condition of the host with the parasite.

In the course of collection and studies of fungi in several parts of India, often in sweltering heat, the writers have found the use of leaf sheath of banana extremely useful. The aerial part of the plant is a pseudostem covered with the sheathing leaf bases. These sheathing leaf bases are green outside, succulent and boat-shaped. By folding on either side, a hollow chamber is formed in which the specimens can be placed (FIG. 1). By firmly tying with a string, the margins of the sheath come together and form a close moist chamber. Fresh plant materials when collected and placed inside this have remained in a fresh state for over 12 days in spite of the rough conditions during transit by postal or railway parcel. Material when taken out at the end of this long period appears as fresh as if collected a few minutes before. To test the efficacy of this method, diseased plants affected by bacterial plant pathogens were despatched by railway parcel. At the end of 10 days the material was found to be in a very fresh condition, enabling the isolation and cultural study of the pathogen. By employing this method, material may be despatched from Puerto Rico to Wisconsin or from the Gold Coast of England by parcel post and received at the destination in a fresh condition to enable critical studies.

4. BANANA LEAF SHEATH CHAMBER FOR INOCULATING EXCISED LEAVES OF PLANT PATHOGENS

The petri dish method of inoculation described by Clinton and McCormick (**1924**) for inoculating excised leaves with rust spores is used in several laboratories. This method enables one to carry out inoculation experiments in cases where potted plants of the host are not available and also ensures protection against contamination from air-borne spores. The longevity of the leaves under the conditions prevailing in the petri dishes varies from plant to plant, some of the thin-leaved plants being susceptible to early decay. In the present studies, it was noticed that in every case the banana leaf sheath supported the host leaves in a fresh condition for 5 to 6 days more than the petri dishes during the same period of incu-

bation. It was therefore found useful to use the banana leaf sheath chamber for incubating excised plant leaves and shoots after inoculating them with the required plant pathogen. This method has been found very useful in carrying out inoculation studies and obtaining critical data even in remote field stations where the usual laboratory facilities are not available.

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SAMUEL CHESTER DAMON II

WALTER H. SNELL

(WITH PORTRAIT)

Samuel Chester Damon II was born in Baltimore, Maryland, on November 18, 1923, the son of Samuel Reed and Jeannie Reid Damon. After completing his high-school education at Towson, Maryland, and Montgomery, Alabama, he entered Brown University in 1940 and remained until the fall of 1943, receiving his B.A. degree with honors in Botany, with the class of 1944.

After one day in the army, he received his discharge in order to allow him to enter the navy and he was commissioned as an Ensign early in 1944. His indoctrination was followed by training at the Bomb Disposal School in Washington and he served as bomb-disposal officer in the South Pacific. He completed his service on August 1, 1946, as Lieutenant (j.g.), with the following authorized decorations: American Area Campaign Medal; Asiatic-Pacific Area Campaign Medal (2 stars); European-African-Middle-Eastern Area Campaign Medal; Philippine Liberation Ribbon; World War Two Victory Medal.

As an undergraduate, Damon, like most young men of the period, was unsettled as to his professional future. He took his concentration in botany and bacteriology but could demonstrate no particular liking for anything in either field at that time. The best interest he could show in mycology was to honor the author of this article by auditing his lectures. During his war service, however, he developed an enthusiasm for medical mycology and returned to Brown in the fall of 1946 for graduate work in mycology. In this period, he took a short course in medical mycology with Dr. Norman F. Conant at Duke University. Even though this special field continued to be a mycological avocation with him, for his two graduate theses he investigated certain imperfect fungi. He was awarded the Ph.D. degree by the University of Iowa in 1951

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after three years of study under Dr. G. W. Martin. He began his professional work immediately as Taxonomic Mycologist in the Research and Developmental Laboratories of the Philadelphia Quartermaster Depot of the U. S. Army.

Damon was married to Miss Meffie Scales of Iowa City in April, 1949, and a son, Samuel Reed Damon III, was born to them on January 28, 1950.

Toward the end of his graduate work at Iowa, he underwent a minor gland operation which could have been only a minor ailment. It was not long, however, before the trouble proved to be more insidious—Hodgkin's disease. He soon realized that his time was limited and that his work was to end in the near future. Nevertheless, he faced the facts with indomitable courage and true bravery—at times almost with detachment, it seemed, as he followed in detail the progress of the disease—planning further research and working doggedly each day to the limit of his waning strength, until the moment he was compelled to return to his family home in Kingston, R. I., for the end he clearly foresaw. In a little more than a year after the onset of his disease, he died, in Kingston, on August 8th, 1952.

This young man was endowed with a clear and keen mind, a high degree of intelligence, and an excellent critical sense. He not only had broad scientific interests and training but he had a wide acquaintance with literature and music, and his knowledge of history provided a discerning understanding of current events. He had a well developed bump of scientific curiosity. At times, apparently interested in nothing in particular and making no noticeable effort, he would suddenly turn up with the most unusual and recondite material from the strangest places, and all in such time that one would wonder not only when but where and how he obtained it. It never paid to enter an argument with him on any subject without being quite certain of one's facts. He was accused of working all night every night but libraries do close and the present writer happens to know the hours kept by this young man.

The lighter side of Sam Damon's character was most unusual and fascinating. He was a jovial companion, with a delicious sense of humor. His enjoyment was expressed variously with a

sly twinkle, a quiet and throaty chuckle or with head thrown back, a contagious guffaw of laughter from the depths. He loved to josh, even rather violently at times, with his best friends; he enjoyed to the utmost being ribbed as well as ribbing. He delighted in assuming deceptive attitudes and postures, giving wrong impressions to the uninitiated. He cultivated an air of extreme cynicism. He habitually portrayed himself as an apostle of laziness and extolled the virtues of laziness as a manner of genteel living. With pipe in mouth, feet on desk, lids half closed over keen eyes—a picture of complete relaxation—he appeared to be doing little but loafing leisurely and accomplishing nothing by the hour, but the wheels were always turning and the results produced were astounding, albeit in no spectacular manner.

To those of us who knew him well, it will be long before anyone can fill his place in our laboratory, because of his keenness behind a feigned stupidity, his seriousness disguised by a superficial frivolity, his accomplishments in spite of his pretended laziness and seemingly lackadaisical responses, the soundness and solidity of his character, and his deep loyalty to persons and institutions beneath an assumed cynical and even flippant attitude. It will be long before we have another student so enjoyable and gratifying personally, socially and academically, and with such obviously uncommon promise for mycology as Sam Damon.

His herbarium, mostly of Fungi Imperfecti, and including 9 types, has been presented to the New York Botanical Garden.

He was a member of the Society of the Sigma Xi, the Mycological Society of America, and American Association for the Advancement of Science, the Torrey Botanical Club, and the Scandinavian Society for Plant Physiology. The results of his scientific work were published or are projected as follows:—

(with Walter H. Snell) Two unusual hosts. Plant Dis. Rep. 32, **10**: 447. Oct. 15, 1948.

A taxonomic consideration of two cheirosporous genera, *Cheiromyces* and *Pedilospora*. Mycologia **42**: 554–562. 2 figs. 1950.

Two noteworthy species of *Sepedonium*. Mycologia **44**: 86–96. 1 fig. 1952.

Type studies in *Dictyosporium, Speira,* and *Cattanea.* Lloydia **15**: 110–124. 2 figs. 1952.

On the fungus genera *Titaea, Monogrammia,* and *Araneomyces.* Journ. Washington Acad. Sci. **42**: 365–367. 1952.

Notes on the hyphomycetous genera *Speggazinia* Sacc. and *Isthmospora* Stevens. Torrey Bot. Club Bull. **80** (In press).

Type studies of certain genera of the Hyphomycetes (In ms.).

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NOTES AND BRIEF ARTICLES

A Simply Prepared Identification Medium for Candida albicans [1]

In routine laboratory procedure, *Candida albicans* is usually distinguished from the non-pathogenic species of the genus by the formation of large, thick-walled chlamydospores on corn meal agar. This characteristic suffices for routine identification, and usually renders unnecessary the more elaborate fermentation reactions and rabbit inoculation (Conant *et al.*, Man. Clin. Mycol. 1944).

The main purpose of corn meal agar is to provide a medium poor in nutrients which favors the production of chlamydospores. The preparation of this medium is, however, both laborious and time-consuming (Lewis & Hopper, Int. Med. Mycol. ed. 3. 1948).

The following identification medium is easily and quickly prepared and induces the formation of chlamydospores as satisfactorily as corn meal agar.

Required ingredients:

White rice	20 grams
Tap water	1000 ml
Agar	20 grams

Cover the rice with the water and bring to a boil. Allow to boil over a low flame for 45 minutes, keeping the vessel covered.

Then filter the liquid through gauze and allow it to cool. Discard the rice.

[1] From the Department of Dermatology and Syphilology of the New York University Post-Graduate Medical School (Dr. Marion B. Sulzberger, Chairman) and the Skin and Cancer Unit of the New York University Hospital.

After cooling, add tap water to the liquid to make 1000 ml. Add 20 grams of agar, and heat the mixture over a low flame or in a water bath until the agar is dissolved.

Sterilize the medium for 15 minutes at 17 pounds pressure and 120°C and pour into sterile Petri plates, or measure into test tubes before sterilizing, sterilize, and slant.

Chlamydospores were observed consistently and in abundance 17 to 24 hours after inoculation of this medium with *Candida albicans*. The morphology of both the pathogenic organism and the non-pathogenic Candidas agreed in every respect with that of the cultures usually obtained on corn meal agar.

With the addition of 2% dextrose C.P. this rice infusion medium may also be substituted for corn meal in the differentiation between *Trichophyton mentagrophytes* and *Trichophyton rubrum* (Bocobo & Benham, Mycologia **41**: 291. 1949).—CLAIRE L. TASCHDJIAN.

A NEW NAME FOR VELUTARIA RUFO-OLIVACEA

It is now apparent that no valid generic name is available for *Velutaria rufo-olivacea* (Alb. & Schw. ex Fr.) Fckl. (and its allies) so long as the present International Code of Botanical Nomenclature (Regnum Vegetabile **3**: 49, 50. 1952.) requires us to accept *V. griseo-vitellina* (Fckl.) Fckl. as the lectotype of the genus *Velutaria*. I earlier (Mycologia **45**: 296–301. 1953.) showed that this typification would be required, but stated that the generic name *Phaeangella* (Sacc.) Sacc. & Sacc. (Syll. Fung. **18**: 128. 1906.) might be available for the *rufo-olivacea* group, provided the lectotype selected by Petrak (Sydowia **3**: 199. 1949.), *P. aceris*, should prove to be a synonym and also to be the first lectotype designated. However, I overlooked the fact that Massee (Brit. fungus-fl. **4**: 136. 1895.) had raised Saccardo's subgenus to generic rank eleven years earlier. He included only three species in the genus, and no type was designated, but of these only *P. ulicis* (Cooke) Massee was included in *Cenangella* subgen. *Phaeangella* Sacc., and is thus the only species eligible as the type of the genus *Phaeangella* (Sacc.) Massee. According to Nannfeldt

(Nova Acta Reg. Soc. Sci. Upsal. 4, **8**(2) : 83. 1932.) this species is probably a *Dermatea* (*Dermea*) or a *Pezicula*.

Unless the rules are changed to take into account predominant usage, *"Velutaria" rufo-olivacea* is left without a valid generic name, or the name must be conserved. While a number of species have been described in this genus (sensu auct. non Saccardo), only one is at all certainly distinct. It is difficult to make a good case for the conservation of such a small (probably monotypic) genus, and I have abandoned hope of effecting such conservation. I propose, therefore, a similar generic name, **Velutarina** nom. nov., to replace *Velutaria* Fuckel, Symb. myc. 300. [1870.] *senus* Rehm *in* Rabenh. Krypt.-Fl. **1**(3) : 645. 1892 (non *emend.* Sacc. Bot. Centralbl. **18**: 219. 1884.; Syll. Fung. **8**: 488. 1889.). The type species of the genus is **Velutarina rufo-olivacea** (Alb. & Schw. ex Fr.) comb. nov. (basonym: *Peziza rufo-olivacea* Alb. & Schw. ex Fr. Syst. myc. **2**: 99. 1822.).

The generic name *Velutaria* Fuckel *emend.* Sacc. must be retained as a genus of the Hyaloscyphaceae, with its type species *V. griseo-vitellina* (Fckl.) Fckl. *Tapesina* Lambotte *emend.* Höhn., as used in my monograph of the Arachnopezizeae (Lloydia **14**: 129–180. 1951.), falls into synonymy.—RICHARD P. KORF.

DISPOSITION OF NOMINA GENERICA CONSERVANDA PROPOSITA FOR FUNGI

As a result of a second ballot on the original proposals concerning *Tricholoma* (cf. Mycologia **45**: 321), the following was approved by the Special Committee for Fungi, and should be added to the list of proposals adopted under paragraph V (p. 317):

Tricholoma (Fr.) Kummer, Führer Pilzk. 25. 1871.—T.: *Agaricus flavovirens* A. & S. ex Fr., Syst. Mycol. **1**: 41. 1821 (cf. *A. equestris* Fr., Elench. Fung. **1**: 6. 1828).

Tricholoma Benth. in DC., Prodr. **10**: 426. 1846.—T.: *T. elatinoides* Benth., l.c.

—DONALD P. ROGERS, Secretary, Special Committee for Fungi.

REVIEWS

Les Agaricales II. Russulacées, Hygrophoracées, Gomphidiacées, Paxillacées, Boletacées, by P. Konrad and A. Maublanc. (Encyclopedie Mycologique XX); P. Lechevalier, Paris. 202 pp., unbound, Price 2200 francs.

The second volume of this work follows rather closely the general organization of material adopted for the first volume. It treats the families mentioned in the title as opposed to the entire remainder of the Agaricales which were combined in the first volume as Agaricaceae. The Hygrophoraceae and Gomphidiaceae consist exclusively of their respective type genera; *Paxillus* and *Phylloporus* form the Paxillaceae, and the Boletaceae are subdivided in two subfamilies (Boletineae and Strobilomycetineae) and 10 genera. The authors have continued to follow a middle course between modern and conservative concepts, an attitude that may have its educational merits, for this work will undoubtedly have influence on the shaping of taxonomical thought of amateurs as well as beginners for which the work is mainly intended. The presentation of a "middle of the road" classification will tend to introduce the progress of systematics during the last thirty years in a gentle, easily digestible and prudently eclectic manner. The book gives a generally true picture of what the average of the mycologists of the foremost country in agaricology, France, is willing to accept at present. The high standard of French mycology, best illustrated by the series of publications in Lechevalier's Encyclopédie Mycologique which has produced such classics as Heim's *Inocybe* monograph and Kühner's *Mycena* studies, is maintained in volume XX of this collection here reviewed. The enumeration of the species is up to date and reasonably complete, and the short diagnoses in the Linnean spirit are useful for a fuller understanding of the relative position of each species. The American reader will find the work useful as a synopsis of the European agaric and bolete flora and will use it for this purpose together with Pilát's

recent "Klic" (Key); he will, however, perhaps more than the European reader, wonder about the scarcity of references to non-European literature on the subject, and the multitude of cases of disregard for the rules of nomenclature. Earlier specific epithets are often given as synonyms (*Boletus aeruginascens, Boletus floccosus, Boletus pseudoscaber, Pluteus atromarginatus* (Konrad) Sing.), and the quotation of the author is very frequently inexact. The consultation of American type studies would have avoided misinterpretation of such species as *Russula pectinatoides, R. ochroleucoides, R. turci, R. neglecta*, etc. These examples could easily be extended, yet it may be said that Konrad and Maublanc's work is rather less remarkable for these tendencies than that of other contemporaneous European writers. The minor shortcoming just mentioned cannot distract from the conclusion that Konrad & Maublanc's Agaricales should be part of every mycological library.—ROLF SINGER.

RUSSULA-MONOGRAPHIE (DIE PILZE MITTELEUROPAS, BAND III), by Julius Schäffer, Verl. Julius Klinkhardt, Bad Heilbrunn, Austria. 296 pp., 20 plates, price 7 pounds 10 sh.

The book consists of a text volume which is a reedited copy of the original Russula-Monographie (Annales Mycologici **31**: 305–516, 1933 and **32**: 141–243, 1934) and the plates partly published as vol. III of the Pilze Mitteleuropas and now added to the revised text. The work is, consequently, partly posthumous, and the revision consists merely in the editing of later published and unpublished notes left by Schäffer and inserted by A. Flury, Basel. The republication of the original monograph is in itself of questionable usefulness, but may be justified insofar as it was apparently considered necessary to add some finished text to the plates. The additions, however, have been edited very poorly and the new text contains many more printing errors than the original. Naturally, many papers have been published on *Russula* between 1933 and 1952, yet they were neither entirely ignored, nor were they fully taken into consideration. Thus, *Russula ferreri* Sing. was introduced although Schäffer never knew it and no plate is available; on the other hand, there is no trace of important new species like

R. chamaeleon Sing., or the results of new type studies as on *R. gracilis* Burl. (*R. gracillima* J. Schäffer), *R. blackfordiae* Peck (*R. versicolor* J. Schäffer), *R. pulchella* Borszcz. (*R. palustris* Peck, *R. exalbicans* (Secr.) sensu Melzer & Zvára, *R. depallens* Fr., sensu J. Schäffer), *R. mariae* Peck, etc. Even the editing of the unpublished notes has been done carelessly; one of the most obnoxious cases of this sort is the verbatim reprinting of the description of *R. olivascens* (no. 38) and the inserting of an added species, *R. chamaeleontina* (no. 41) whereby the text reveals that J. Schäffer's real intention was to combine the two species under the name *R. chamaeleontina,* which is obvious because of the fact that *R. olivascens* is indicated as a synonym of *R. chamaeleontina.* The reviewer as well as the majority of European specialists does not agree with this opinion, as *R. chamaeleontina* is obviously an outstanding example of a nomen dubium and a nomen ambiguum, and the identity of R. no. 38 and no. 41 is improbable, yet the point is that the editor did not understand Schäffer's position and the result is an inconsistency which goes through the entire book. While it is still argued that *R. raoultii* Quél. is "unterbestimmt" (not completely known)—p. 218—or systematically valueless (p. 216), the species must have had some meaning to the author since a good colored picture is given in the second volume. The additions to the keys (pp. 262–288) are equally unfortunate. *R. cremeoavellanea* is wrongly inserted among the species with the context turning blackish gray and is entirely absent in the "Synoptische Tabellen." There is no use in emphasizing the points on taxonomy or nomenclature in which this reviewer and Schäffer disagree; they have been discussed in various publications, and should not be rediscussed on this occasion. In spite of their shortcomings, Schäffer's views have found a considerable degree of acceptance in European publications, and this tendency will perhaps grow as long as Schäffer's monograph is the latest and most easily available contribution to the knowledge of the genus. However, it must be stated that the present edition of the monograph does not contribute much to the general knowledge of the Russulae, and rather confuses some of the issues. On the other hand, the illustrations are very valuable and it is indeed helpful, especially for

the beginner, to have another atlas added to those already existing in Europe, such as Melzer's excellent but smaller "Atlas Holubinek" (Praha 1945). In quality of design, color, and reproduction, Schäffer's figures are undoubtedly superior to most other publications containing European Russulae, and the fact that these plates actually represent the majority of Europe's Russula flora contributes greatly to their value.—R. SINGER.

SOIL MICROBIOLOGY, by Selman A. Waksman. New York: John Wiley and Sons, Inc. 356 pages (illus.), 1952. Price $6.00.

This book by a noted authority in the field of microbiology was designed as a textbook. It includes considerable material, including tables and illustrations, found in two previous books, Principles of Soil Microbiology (1927), and The Soil and the Microbe (1931). As indicated in the preface the aims of this book were "to survey the nature and abundance of microorganisms in the soil, to review the important role that they play in soil processes and, as far as possible, to show the relation between them and soil fertility."

Although reference to and discussion of many investigations conducted in various parts of the world are presented, much of the material in the book naturally had its origin in the extensive researches of Doctor Waksman and his associates. It is not claimed by the author that all phases of the subject of the soil microflora are covered in a comprehensive manner. The systematic mycologist may, in fact, find a paucity of information on his favorite subject.—O. H. SEARS.

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OBSERVATIONS ON STREPTOMYCES GRISEUS. VI. FURTHER STUDIES ON STRAIN SELECTION FOR IMPROVED STREPTOMYCIN PRODUCTION [1]

EUGENE L. DULANEY

(WITH 1 FIGURE)

The use of induced mutation and strain selection to improve yields of a product produced by a microorganism is now well recognized. The marked increase in penicillin production that resulted from the application of this approach has been well documented (**1**, **4**). Thus far, however, nothing has been published concerning the use of this method to improve yields of other antibiotics with the exception of streptomycin, and even in this instance, the data are meager.

Stanley (**6**) used strain selection to improve streptomycin yields and reported maximum broth potencies of 800 γ/ml. Savage (**5**) reported the isolation of strains that produced activities of 1,000 γ/ml, but noted their marked instability. Dulaney, Ruger, and Hlavac (**2**) published on the isolation of mutants capable of producing 320% more streptomycin than the starting culture.

[1] Contribution from the research laboratories of Merck & Co., Inc., Rahway, New Jersey.

[MYCOLOGIA for May–June (**45**: 325–480) was issued June 22, 1953]

The present paper is a further report on our isolation of *Streptomyces griseus* mutants with an increased capacity for streptomycin production and presents scientific details of experiments which have led to the high-producing strain.

EXPERIMENTAL METHODS

The starting culture for this work was Waksman's No. 4 isolate of *Streptomyces griseus*. Stock cultures of this strain and of the mutants to be described later were prepared by drying spores in sterile soil. As cultures were needed, spores were transferred from the soil to slants of yeast extract-glucose agar or to an agar medium with a soybean meal-distillers solubles base. Changes in the medium used for developing spore crops were made necessary by the differences in sporulating capacities of the various strains. Spore crops were harvested by washing seven-day-old cultures incubated at 28° C with sterile, distilled water and filtering the resulting spore suspension through several layers of sterile absorbent cotton. This filtrate was subjected to various mutagenic agents, but only ultraviolet light and x-rays are pertinent to the results to be reported. In practice, exposure of the spores to the mutagens was for a time sufficient to kill approximately 99% of the spores. The treated suspensions were plated on a medium that supported good sporulation of the strain in question, and the plates were incubated at 28° C until good growth and sporulation occurred. Colonies thus developed were transferred to agar slants, incubated until sporulation was satisfactory, usually seven days, and either tested at once or stored at 4° C until used. It should be noted that there was no selection of the colonies for testing, but isolation of colonies was at random with all colonies on a plate usually being tested.

As work progressed, the experimental conditions used for screening the isolates changed. At first, a spore suspension was used as inoculum. This was later changed to vegetative growth developed on rotary shakers for 48 hours in a medium composed of glucose 1%, sodium chloride 1%, N-Z-amine 1%, meat extract 0.6%, and distilled water to volume. A number of other media would serve just as well. In all instances, however, one ml of

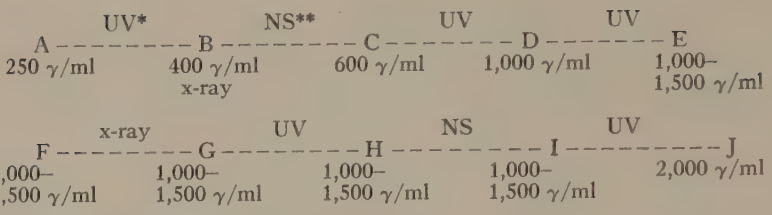
either spores or vegetative growth was used to inoculate a 40 ml volume of medium in either 125 ml or 250 ml Erlenmeyer flasks. The smaller flasks were used earlier in the work, but a change was made later to the larger size. These changes did not markedly affect the maximum broth potencies obtained but did somewhat change the time at which maximum activities occurred. The flasks were incubated at 28° C on rotary shakers moving at 220 rpm and describing a circle approximately one inch in diameter.

Whole broth samples, taken at appropriate intervals, were diluted in M/20 phosphate buffer and assayed by the cylinder-plate method using a reference standard of streptomycin sulfate or the calcium chloride complex.

With progressive isolation of improved strains, it was found to be necessary to change the concentration of some of the ingredients used in the screening medium. The medium used at the outset of these experiments, described in our earlier publication, was composed of soybean meal 2%, glucose 1%, sodium chloride 1%, and distilled water to volume. Following the isolation of each improved strain, it was found to be necessary to increase the level of nutrients in order to obtain maximum production. The subsequent use of a medium containing distillers solubles, as reported by McDaniel (3), resulted in improvements in the yield of streptomycin by the *S. griseus* cultures described in this paper. A medium used successfully for testing the high-producing mutants contained soybean meal 4%, glucose 2.5%, distillers solubles 0.5%, sodium chloride 0.25%, and distilled water to volume.

DISCUSSION OF RESULTS

A marked increase in streptomycin productivity has been accomplished by induced mutation and strain selection. Data obtained in laboratory experiments, illustrative of the type of improvements which can be demonstrated when working with small scale fermentations, are summarized in FIG. 1. The original culture, strain A, produced 250 γ/ml. This was a single colony isolate of the Waksman No. 4 strain. Since initiation of growth from spores was inhibited by streptomycin, it was made resistant to a streptomycin concentration of 600 γ/ml by serial passage in



*UV—ultraviolet light
** NS—natural selection

FIG. 1.

media containing increasing levels of streptomycin. It was feared that the sensitivity of the culture to streptomycin might be limiting the productivity of the culture. The study of development of resistance to streptomycin has been pursued no further, however, and cultures producing considerably in excess of 600 γ/ml have been obtained. If sensitivity to streptomycin has any significance in the isolation of superior strains, then we have been selecting for streptomycin resistance, as well as for streptomycin production. The testing procedures employed in the latter stages of this study, including the use of vegetative inoculum, are not conducive to the demonstration of streptomycin sensitivity in the mutant cultures.

Spores of strain A were subjected to ultraviolet light treatment, and strain B, capable of producing 60% more streptomycin than strain A, was thus obtained. In the beginning a great deal of exploratory work was concentrated on this culture which is also strain B of our previous publication (2). An isolate, strain C, which was 50% better than strain B and 140% better than strain A, was obtained from strain B by single-colony isolation. Strain C, strain G of our previous paper (2), was subjected to ultraviolet-light treatment, and strain D was obtained. This strain was listed as strain I in the previous publication (2) where it was reported as producing 3.2 times as much streptomycin as strain A. Actually it has been found that four times as much can be obtained by medium modification.

Strain D proved to be quite stable, but by induced mutation, markedly superior isolates were obtained from it. Strain E, which yielded 50% more streptomycin than strain D, was obtained from

strain D, following treatment with ultraviolet light. Strain F was obtained by subjecting strain E to x-radiation, and further x-radiation of strain F yielded strain G. By treatment of strain G with ultraviolet light, strain H was obtained, from which strain I was isolated by single-colony selection without treatment of the spores with a mutagen. All of these strains, namely, E, F, G, H, and I, were 50% improved over strain D, the highest producer described in previous publications. All the cultures were quite unstable, however, and after several transfers degenerated to production levels of their parent strain. This loss of productivity can best be ascribed to genetic instability or to heterocaryosis.

Strain I was subjected to treatment with ultraviolet light, and strain J was obtained. This culture is capable of producing relatively high yields of streptomycin and unlike its immediate progenitors is quite stable in relation to streptomycin productivity. Survival curves of this strain, following treatment with ultraviolet light, were somewhat sigmoid; whereas, survival curves of strains A and D were exponential. These sigmoid curves are indicative of multinucleate spores and indicate the possibility of heterocaryosis as the cause for the instability in strains E, F, G, H and I. It should be noted, however, that no stable high-producing homocaryotic races could be isolated from these strains by single-colony selection. In addition, if these strains were heterocaryons, their degeneration to a stable low production level indicates that the mutant nuclei were being selected against in the cytoplasm under the experimental conditions being used. Perhaps a change in the medium or in some other experimental factor would have induced a selection in favor of the mutant nuclei.

It should be emphasized that the values given in FIG. 1. were obtained through comparative tests under a given set of conditions. The most pertinent of these conditions was the use of the concentrated medium noted in the section on experimental methods. This point is emphasized, for some of these strains have been found to produce broth potencies different from those listed in FIG. 1 when tested with various fermentation media.

It should be realized that screening cultures under a given set

of conditions may result in cultures being selected that produce maximum broth potencies only under the screening conditions.

It is not always true, however, that a mutant capable of producing increased yields of a compound under the selecting conditions will produce its maximum yield only under those conditions. Hence, each successive mutant should be carefully studied in order to determine the conditions optimum for its productivity. For example, strain J is listed in FIG. 1 as producing eight times as much streptomycin as strain A. However, slight changes in such physical factors as aeration, agitation, and incubation temperature resulted in this strain producing a further increase in broth potencies of 15%.

The question arises as to the reason for increased productivity by these mutants. Perhaps increased productivity is due to a favorable change in some enzyme system operating in the synthesis of the limiting moiety of the streptomycin molecule. Perhaps this increased productivity is due to an enzymatic block that shunts intermediates from one path to the formation of streptomycin. It is also possible that the mutation results in the ability of the strain to overcome some inhibitor present in such a complex medium. In the successive selection of a number of mutants, a multiplicity of factors may be involved. Several of these mutants have been studied in synthetic media in an attempt to find physiological differences that might relate to the differences in productivity of streptomycin. The only difference found, however, was the ability of certain strains to grow efficiently and produce increased streptomycin broth potencies in a synthetic medium with L(-)proline as the sole nitrogen source. Strains B and C were markedly superior to strain A in such a medium, and strain D was far superior to strains C and B. None of the strains from E to J, however, were superior to strain D in the proline medium.

SUMMARY

Through the use of ultraviolet light, x-rays and natural selection, mutants were obtained that were capable of producing markedly higher streptomycin broth potencies than the starting culture. The highest streptomycin activities observed were over eight times

more than the broth potencies produced by the starting culture, Waksman No. 4, under the same experimental conditions. Many thousands of cultures were examined in the selection of cultures described in this paper.

The selectivity that often results from the experimental conditions used in screening cultures is emphasized.

MERCK & CO.,
RAHWAY, NEW JERSEY

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THE EFFECTS OF IONIZING RADIATIONS FROM POLONIUM ON THE SPORES OF ASPERGILLUS NIGER [1]

SIGMUND BERK [2]

(WITH 28 FIGURES)

The literature on the effects of ionizing radiations on fungi has recently been reviewed by Berk (**1952a, b**). More pertinent references will be listed in this article.

Biological effects are produced by many types of radiation if the dose is great enough. Of these, ionizing radiation is responsible for many radiobiological effects. The massive nuclear fragment known as the alpha particle not only makes direct and glancing collisions with atoms and molecules but also acts as a very efficient ionizer during the "raying" process. Stapleton *et al.* (**1952**) reported that alpha particles are more efficient than X-rays in inactivating *Aspergillus terreus* spores.

Ionizing radiations are injurious to cells that absorb them. Gray (Uber, **1950**) reported that the radiation interfers with almost every known function of the cell when delivered in sufficient dosage. There is an increase in the accumulation of evidence that ionizing radiation destroys enzymes and interferes with the carbohydrate metabolism of the cell. Barron (**1950**) found that ionizing radiation oxidizes the sulfhydryl groups of the cell.

In a previous article (Berk, **1952b**) it was reported that the alpha particle irradiation of *Aspergillus niger* spores in culture medium produced mutants of the fungus. The biological effects

[1] Extract presented before the Society for Industrial Microbiology meeting held in conjunction with the American Institute of Biological Sciences at Cornell University, Ithaca, New York, September 9, 1952.

[2] The author wishes to express his appreciation to the Ordnance Corps of the Department of the Army for permission to publish this article. Special thanks are due to Dr. R. E. Zirkle of the Institute of Radiobiology and Biophysics of the University of Chicago for his helpful comments.

produced were contributed not only by the ionization of the fungus but also by the ionization of the components in the culture medium. In this study dry conidial spores were irradiated with varying doses of alpha particles from polonium and the spores were then germinated on culture medium. An attempt was made to correlate dosage of alpha radiation with inhibition of biological processes in the germination and growth of the fungus.

EXPERIMENTAL METHODS

A single spore isolate from a culture of *Aspergillus niger* van Tiegh. USDA TC *215–4247* was used (Berk, **1952b**). This single spore isolate was designated as FA *300*. Spores harvested from cultures grown on Difco potato-dextrose agar for 3 to 6 days were used to prepare the spore suspensions.

A small drop of the aqueous spore suspension was placed on glass microscope slides which had small circles scratched on the under surface of the slide to facilitate the microscopic examination of the spores. Littman-Well slides were also used (Littman, **1949**). The spore drops were allowed to dry at room temperature and then placed above the radioactive source for irradiation. The slides were then removed from the radioactive source and a drop of melted (40°–45° C) potato-dextrose agar diluted with two parts of distilled water added to the irradiated spores. The slide cultures were incubated over water in 10 cm. desiccators for 17 hours to 6 days in a room maintained at 29° ± 1° C. The 0.5 per cent agar in this culture medium remained in a liquid state at 40° C but solidified immediately upon contact with the microscope slide containing the spores. At the end of the incubation period the thin films of agar formed a flat surface for the mounting medium and cover glass.

After 17 to 144 hours of incubation, a drop of Amann's lactophenol containing cotton blue as a stain was added to the slide. The spores were examined with the compound microscope at a magnification of × 440.

GERMINATION IN ABSENCE OF RADIOACTIVE MATERIALS. After four hours' incubation no visible changes were evident in the spores incubated in the absence of radioactive materials. The

FIGS. 1–7.

spores show the first signs of germination after five hours' incubation by swelling from 5 to 7 microns in diameter. After six hours, the spore swells to 7 to 8 microns and the formation of a germ tube (mycelium beak) is visible. FIG. 1 shows the appearance of the swollen unstained spores after 6½ hours' incubation. After eight hours' incubation most of the spores have the germ tube and show formation of hyphae. From 17 to 24 hours' incubation the germinated spores have entensive mycelial growth (FIG. 5) and show the development of foot cells bearing conidiophore initials. In some of the slides the primary and secondary sterigmata with immature conidia attached were visible.

From 25 to 30 hours' incubation, some of the slides showed a complete conidial apparatus with spores. After 48 hours' incubation all germinated spores had conidiophores bearing chains of mature conidia.

IRRADIATION OF SPORES. The slides containing the dry spores were placed above the polonium sources which were shielded with a stainless steel cutout so that a disc 12.7 mm. (½ in.) in diameter was exposed to the spores. The distance between the slide and the alpha particle source was approximately 13 mm.

The polonium samples consisted of nickel foils plated on one surface with varying amounts of polonium. The active side of the foils was coated with a thin gold film, approximately one micron in thickness, to reduce the contamination hazard. The values used in the experiment are averages of the external radiation of two samples of each concentration used. TABLE I lists the calculated activity of the 12.7 mm. diameter discs of polonium at the start and at the finish of the experiments. Eight polonium sources with four activities of polonium varying from 14.7 microcuries (μc.) (5.45×10^5 alpha particles/sec.) to 291 μc. (1.08×10^7 alpha particles/sec.) per disc were used.

The dry spores on the glass slides were irradiated with alpha particles from the polonium sources for lengths of time varying from 20 minutes to 360 hours. Control slides containing spores were exposed in the same 10-inch desiccators that contained the polonium but at a considerable distance beyond the range of the alpha particles.

CALCULATION OF DOSE. The calculation of the dose used is similar to the method described by Zirkle (**1932**).

The total number of alpha particles received by the spores in the experiments ranged from 26 to 74,000. The dose in roentgen-equivalent-physical (rep) received by the spores during these experiments varied from 4800 to 17,000,000.

It is known that the linear energy absorption along the tracks of polonium alpha particles varies in a definite manner (Zirkle, **1940**). Since the range of polonium alpha particles in air is 38.8 mm. and since the range of the alpha particles in tissue having a

TABLE I

INITIAL AND FINAL ACTIVITY OF POLONIUM SOURCES

Source number	Date	Activity in μc. per disc	No. of alpha particles emitted per sec./disc ($\times 10^{-5}$)
1	7/14/49	291	110
1	10/12/49	188	70
2	8/5/49	38	14
2	10/12/49	27	10
3	8/10/49	20	7
3	10/12/49	15	5
4	9/15/49	288	110
4	10/12/49	257	95

density of one is given by Lea (**1947**) as 38.9 μ, 1 mm. of air may be considered equivalent to 1 μ of tissue. The radius of the *Aspergillus niger* spore was taken as 2 μ and the distance from the polonium source to the spore was 13 mm. of air, then the linear energy absorption of the spore in this experiment was approximately 1.3×10^5 electron volts per micron (Zirkle, **1940**). If the distance between the polonium source and the spore had been increased, the linear energy absorption by the spore and also the biological effectiveness of the radiation would have increased. Only a small part of the entire track of the alpha particle is spent in the fungus spore.

BIOLOGICAL EFFECTS ON THE IRRADIATED SPORES

Irradiation of the dry fungus spores with alpha particles produced a number of biological effects when the spores were germinated on the culture medium. In this study observations were concerned primarily with the following inhibitions: Swelling of spore, germ tube formation, vegetative growth, and sporulation.

Zirkle (1952) irradiated *Aspergillus terreus* spores with alpha particles. He found that the alpha particle survival curves were exponential when the criterion of survival was the percentage of germ tubes formed from the total number of swollen spores.

When the percentage survival of the irradiated spores was plotted against the dose (expressed either in number of alpha particles or reps absorbed by the spore) on semilog paper, a straight line was not obtained. The nature of the survival curve was such that only approximate values could be stated for the inhibition of the various biological processes. The susceptibility of individual spores to radiation is known to vary. Stapleton and Hollaender (1952) reported that there is a definite raltionship between the X-ray sensitivity of spores of *Asperigillus terreus* and their relative water content during the time of irradiation. It is possible that in these studies the water content of the spores may have varied over a wide range since the aqueous drops of spore suspension were air dried at room temperatures with different relative humidities.

TABLE II lists a number of experiments which show the effect of increasing doses of alpha radiation on the percentage of swollen and germinated spores.

The destructive action of the radiation is exerted by the energy absorbed by the spore. It was found that when the spore received less than 100 alpha particles (23,600 rep) no visible gross effects on germination, vegetative growth, and reproduction of the fungus were noticed.

FIGS. 2 to 28 consist of a number of photomicrographs showing the effect of increasing dosage of alpha particles on the germination, vegetative growth, and reproduction of the fungus. As the number of alpha particles received by the spore is increased from approximately 73 to 8000, the amount of vegetative mycelial

growth is decreased and the number of abnormally germinating spores and lethal mutations is increased. The spores which received 73 alpha particles (13,400 rep) produced spore heads with a complete conidial apparatus after 40 hours' incubation. With low dosages of alpha particles there was such a heavy mycelium growth after 17 to 24 hours' incubation that detailed observations were obscured. FIG. 2 shows that some of the spores which were

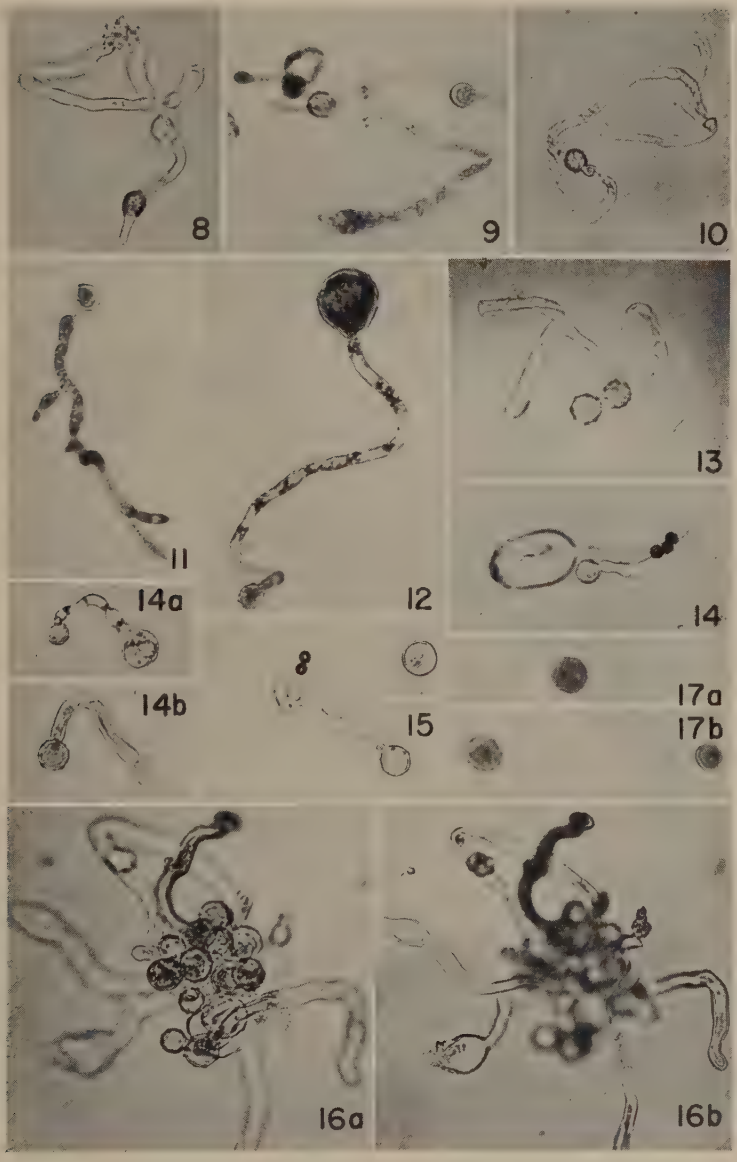
TABLE II

EFFECT OF INCREASING DOSAGE OF IONIZING RADIATION FROM POLONIUM ON THE GERMINATION AND SWELLING OF ASPERGILLUS NIGER SPORES

The irradiated spores were incubated on diluted potato-dextrose agar

Polonium source	Dosage, per spore		Incubation of irradiated spores in hours	Per cent spores germinated	Per cent spores swollen	Per cent spores not germinated
	Alpha particles	Rep $\times 10^{-3}$				
1a	140	26	22	92	0	8
1b	200	37	20	72	0	28
3a	360	66	120	69	8	23
4a	460	85	72	63	7	34
3a	550	101	120	57	0	43
2a	630	116	144	47	20	33
3b	1,000	184	96	30	6	64
2a	1,100	202	96	24	10	66
3a	2,000	368	96	29	23	48
2a	2,400	442	20	25	75	0
2a	3,600	662	96	22	29	49
1a	3,900	718	48	12	26	62
1a	4,300	791	50	6	40	54
1a	4,600	846	120	23	53	24
1a	5,800	1,067	72	12	32	56
2b	6,300	1,159	144	11	37	52
1a	7,000	1,288	120	13	50	37
1b	8,100	1,490	96	2	9	89
4a	22,000	4,048	20	12	66	22
1a	26,000	4,784	96	0	2	98
1a	60,000	11,040	96	0	0	100

bombarded with 108 alpha particles (20,000 rep) produced conidial heads with mature conidia that did not differ from the non-irradiated controls. However, all spores that absorbed in excess of 114 alpha particles (21,000 rep) germinated but failed to sporulate. The first visible biological effect of alpha radiation is inhibition of reproduction or sporulation. This effect is often called genostasis.



FIGS. 8–17.

Spores that absorbed 140 alpha particles (26,000 rep) germinated but had restricted mycelial growth (FIG. 3). An example of the profuseness of the mycelial growth of the control is shown in FIG. 5. The control was exposed in the same desiccator as the spores irradiated with 190 alpha particles (35,000 rep) shown in FIG. 4, and both were incubated for 24 hours. As the number of alpha particles which the spore receives is increased, the vegetative growth beyond the germ tube stage is decreased. It appeared that spores receiving in excess of 114 alpha particles lost the ability to produce a living colony but developed abnormally until all growth ceased.

Ford (**1948**) irradiated *Chaetomium globosum* spores with UV and X-rays and then plated the irradiated spores on malt agar. He found that the spores which germinated grew to a certain point and then growth ceased. To these germinated spores he assigned the name "microscopic mutants." A similar effect was obtained from the alpha irradiation of *A. niger* spores. However, a more fitting name would be "lethal mutants." All spores that received in excess of 114 alpha particles and failed to sporulate may be classed as lethal mutants.

The spores that received 290 alpha particles (53,000 rep) and were incubated for 144 hours (FIG. 7) had hyphae with protoplasmic extrusions which are probably produced by the bursting of the swollen hyphae. There was also an increase in the diameter of the hyphae of the irradiated spores over that shown by the non-irradiated controls (compare FIGS. 6, 7, and 18 with FIG. 5).

Owen *et al.* (**1950**) found that when a species of *Botrytis* was treated with nitrogen mustard gas, the germination time of the fungus was delayed. It was thought that possibly the alpha radiation had a delaying effect on the germination and growth of the irradiated spores. Spores which received 320 alpha particles (59,000 rep) to 600 alpha particles (110,000 rep) had limited growth beyond the germ tube stage, even when incubated for 90 hours (FIGS. 8, 10, 11, 15). The effect of alpha irradiation is more than a delayed action. Continued examination of some germinated spores revealed that they had no more than one growth period. The growth of the fungus ceased shortly after the produc-

tion of a short, thick germ tube which showed little to no branching.

Dimond and Duggar (**1940**) found that when they incubated spores of *Rhizopus suinus* irradiated with UV, there was no appreciable increase in the amount of growth of the germinated spores from 19 to 30 days' incubation. This raised the question of whether germination constituted a reliable measure of survival. Normally, when a spore germinates the fungus is considered to have survived. In observing the growth of the fungus beyond the germination stage, it was observed that the irradiation had effects on the fungus which did not appear until beyond the germ tube stage. Germination of a spore cannot be considered as a true criterion of the survival of the organism. The true criterion of survival is the ability of the irradiated spore to produce a visible colony with reproductive structures (conidial heads) bearing viable spores.

In FIGS. 9, 12, 14 and 16 the spore received from 360 alpha particles (66,000 rep) to 1170 alpha particles (215,000 rep). The hyphal tips of these germinated spores had swellings, club-shaped and sac-like enlargements, and spherical and pouch-like structures. FIGS. 14 and 14a show spores that have swollen germ tubes which terminate in vesicles. Some of these vesicles disintegrated releasing the protoplasmic contents.

As the number of alpha particles received by the spore is increased (*i.e.*, the higher the dose), the greater the number of restricted growth or lethal mutants produced. Ford and Kirwan (**1949**) found that *Chaetomium globosum* spores irradiated with X-rays produced lethal mutants which varied from 1 per cent for a dose of 9.4×10^3 rep to 100 per cent for a dose of 329×10^3 rep. The alpha particle-irradiated spores germinated but showed little growth beyond the germ tube stage. Many spores had a single straggly hypha; others showed branched and unbranched convoluted hyphae. Growth of the irradiated spores occurs up to a certain point and then ceases (FIGS. 13, 14, 15, 19, 20, 25, 27). These restricted growth mutants may be called lethal mutants since the fungus fails to reproduce a viable colony.

In irradiated spores that received in excess of 1000 alpha particles, the number of swollen spores increases (FIGS. 15, 17a, 17b,

FIGS. 18–28.

22, 23, 24). More than 50 per cent of the spores remained in the swollen condition after 20 hours' incubation (FIG. 17). The spore wall of the giant swollen cell fails to break and the formation of a germ tube is inhibited. With these high doses of radiation, growth

is halted at the giant cell stage (FIGS. 17a, 17b). Butler and Rowell (**1951**) exposed *A. niger* spores to colloidal polonium and also found that the spores became excessively swollen and failed to develop further. In FIG. 21, the spores received 2000 alpha particles and were photographed in the unstained condition after 288 hours' incubation on the agar medium.

The spores in FIG. 22 were suspended in a drop of water and irradiated with 3900 alpha particles while the spores were still wet. The number of ungerminated and swollen spores was very high. One swollen spore appears to have exploded and FIG. 22 shows its extruded protoplasmic contents. Henshaw and Francis (**1935**) reported that spores are more resistant to radiation when dry than when moist. No work beyond this single exposure was done on this problem since the spores dried out on prolonged exposure to the radiation, and the activity of the source was not great enough to permit short exposures of the wet spores. Also the thickness of the drop of water was initially great enough to prevent the passage of the alpha particles to the spore.

Some of the spores that were irradiated with 4950 alpha particles had germ tubes that were much inflated and distended (FIGS. 23, 24). Spores that received 7000 alpha particles (1,288,000 rep) to 8000 alpha particles (1,472,000 rep) had a preponderance of giant swollen cells (FIGS. 26, 27, 28) and did not put out a mycelium beak even when incubated for 72 hours. Some of the swollen spores measured 26 μ in diameter. The swollen cells failed to divide and this inhibition of mitosis is one of the effects of high radiation dosages (Catcheside, **1948**). The few swollen cells that did germinate produced a very short germ tube and then ceased growth (FIGS. 27, 28). Some of the germinated spores had coagulated protoplasm and were probably dead. These spores were probably injured to such an extent by the radiation that they were unable to undergo cell division and perished during mitosis. Nonirradiated spores which were exposed in the same desiccator as the spores shown in FIG. 28 produced normal conidial heads after 48 hours' incubation. Armitage and Verdcourt (**1948**) obtained similar results with sodium pentachlorophenate. With a concentration of 40 to 50 ppm the spores enlarged to giant cells

which did not go beyond the spherical stage for 20 days incubation. Luyet (**1932**) found that when *Rhizopus nigricans* spores were irradiated with cathode rays they swell to giant size without ever producing a mycelium. Ting and Zirkle (**1950**) irradiated blood cells with X-rays and came to the conclusion that the swelling of the cells was due to the increase in the permeability of the cell membrane to dissolved substances.

Complete inhibition of swelling and germination was produced by the irradiation of the spores with 25,000 alpha particles (4,600,000 rep) to 60,000 alpha particles (11,040,000 rep). According to Gottlieb (**1950**) the absorption of water varies with the viability of the spore and he considers a spore dead if it does not swell.

Caution must be exercised in generalizing regarding the biological effects of the alpha particles on the dry spores. Nevertheless, an attempt is made to summarize the results obtained. As the dose of ionizing radiation was increased, there was a progressive decrease in the amount of development shown by the germinating spore until finally with large doses they remained inactive. Spores that received low concentrations of alpha particles (less than 50) showed no visible effects, either inhibitory or stimulatory on germination, vegetative growth, or sporulation. All the spores that received more than 114 alpha particles failed to sporulate (reproduce). Initial inhibition of vegetative development appears in spores bombarded with more than 100 alpha particles. Higher doses of alpha particles (1000 +) produce a large percentage of swollen spores and poor germination. Complete inhibition of swelling and formation of a germ tube results from the irradiation of the spore with more than 25,000 alpha particles. The order in which biological processes in the life cycle of the fungus are interfered with in going from low doses of alpha particles to high doses are: (1) sporulation, (2) vegetative growth or cell division, (3) germination (production of germ tube), and (4) swelling of the spore.

GROWTH OF THE IRRADIATED SPORES ON A COMPLETE MEDIUM. Spores bombarded with more than 114 alpha particles failed to sporulate when incubated on potato-dextrose agar. It was thought that the irradiation might have produced biochemical mutants that

could no longer synthesize the essential amino acids, vitamins, and other essential foods from the potato-dextrose agar used as the culture medium. A modification of the culture medium used by Beadle and Tatum (1945) to detect deficiency mutants in *Neurospora* was prepared. A drop of melted medium (45° C) was placed on the nonirradiated and the alpha particle irradiated spores. The nonirradiated control slides had increased vegetative growth and sporulation with the complete medium. No significant improvement in the growth of the irradiated spores was obtained. However, with culture medium containing chemical inhibitors, the effect on the morphology of the fungus is only temporary since normal growth results when the swollen spore is transferred to normal culture medium (Verdcourt, 1952).

Spores irradiated with 140 to 460 alpha particles and incubated on the complete medium did produce a higher percentage of swollen hyphal tips, pouch-like enlargements at the ends of the hyphae, and protoplasmic extrusions than those incubated on potato-dextrose agar. Ford (1948) also found no improvement in the growth of "microscopic mutants" when they were incubated on a more complete medium after irradiation by UV and X-rays. Spores that were irradiated with 32,000 alpha particles failed to swell on the complete medium. This high dose of alpha particles may be considered to be lethal to the spores.

Some of the irradiated spores were also incubated on 3 per cent malt extract. Lindegren (1949) and Hollaender *et al.* (1945) class malt agar as a more complete medium. In our tests, 3 per cent malt extract failed to stimulate sporulation in the irradiated spores.

DISCUSSION

In the study of the particulate radioactive emanation from polonium on the fungus spore, the dosage was based on the entire spore excluding the spore walls. Hansen and Smith (1932) claim that the basic unit of the organism is the nucleus and not the cell (spore). The nucleus has been reported by many as extremely sensitive to radiation. Petrova (1942) found the cell nucleus 700 times more radiosensitive to alpha radiation than the cytoplasm. Although Zirkle (1932, 1949) reported that nuclear elements were

more susceptible to alpha radiation than the extranuclear ones, he also found that high dosages to the cytoplasm would also be lethal to the cell. Failla (**1937**) reports that ionizing radiation delivers its damage straight to the nucleus through the cell membrane, cytoplasm, and nuclear membrane. At the same time, all the molecules traversed through the irradiated cell become ionized.

The calculation of the number of alpha particles absorbed by the spore nucleus is more difficult since information on the exact dimensions of the nucleus is not available. A number of investigators, Whelden (**1938**), Zahl *et al.* (**1939**), reported that the *Aspergillus niger* nucleus is spherical, and is centrally located in the spore. Wakayama (**1931**) worked on the cytology of the sterigma of the fungus and reported it as uninucleate. The *A. niger* nucleus is described as small and as containing two round small chromosomes in the haploid condition. In the absence of information on the dimensions of the nuclear material of the spore, the size of the sensitive region of the spore was assumed to correspond to the dimensions of the spore minus the thickness of the walls.

In determining the percentage of survival for *A. niger* spores irradiated with the alpha particles some difficulty was experienced in choosing the criteria of survival and death. Zirkle (**1932**) also found that the number of alpha particles required to kill the cell depended on what criteria he used for the death of the cell. More recently Zirkle (**1952**) selected swelling as a criterion of viability of the spore and the protrusion of even a short tube from the swollen spore as a criterion of survival of the fungus. Uber and Goddard (**1934**) reported that the shapes of X-ray survival curves is a function of the criteria selected for death and that the survival curves showed rhythmic variation with dosage. The results obtained in this study revealed that ionizing radiations produce a number of inhibitions in the normal growth processes of the fungus. These inhibitions are: reproduction (sporulation), macroscopically visible mycelial growth, germination (production of germ tube), and swelling of the spore. The dosage of alpha particles required to inhibit these biological processes was found to range from 114 to 25,000. The usual index of death is the inability of the spore to produce a macroscopic colony (Lea, **1946**). Others consider the

failure of a spore to swell as the criterion of death. The author agrees with the criteria of death selected by Uber and Goddard (1934) in their work on the effect of X-rays on the fungus *Neurospora*. These criteria are: (1) germination, (2) "positive growth," and (3) presence of normal reproduction and mature conidia. On the basis of these criteria, all spores that received in excess of 100 alpha particles and failed to produce conidia may be considered "dead" or lethal mutants.

The results show that normal growth processes and reproduction are interfered with long before the mechanisms which are responsible for germination and swelling of the fungus spore are destroyed. Very similar biological effects are produced by the irradiation of fungi with alpha particles and X-rays.

The doses required to interfere with cell division were small compared to the lethal dose for the spores. These results are in agreement with the conclusions reached by Spear (1946).

SUMMARY

Dry conidial spores of *Aspergillus niger* were irradiated with varying doses of alpha particles from polonium sources. It was found that the severity of the biological effects is dependent on the dosage or the number of alpha particles directed at the spores.

There is a definite order in which biological processes are interfered with in going from one dose of alpha particles to higher doses. Less than 100 alpha particles (17,400 rep) directed at the spores had no visible stimulatory or inhibitory effects on germination, growth, or sporulation of the fungus. The first visible biological process interfered with is reproduction (sporulation) or production of conidial heads. This inhibition of sporulation occurs when the irradiated spores receive more than 114 alpha particles (21,000 rep). The second process which is inhibited is cell division or the development of an extensive mycelium (vegetative growth). Slight inhibition of vegetative growth begins when the spore receives more than 100 alpha particles. Conidia receiving in excess of 114 alpha particles developed abnormally before all growth processes ceased. As the number of alpha particles received by the spore increases to 1000 (184,000 rep) the extent of

development decreases and the spore loses the ability to develop a living colony. The inhibition of germination or the production of the germ tube is inhibited at about 1000 alpha particles per spore. Above 1000 alpha particles per spore, very few of the irradiated spores develop beyond the swelling stage. A spore that receives more than 25,000 alpha particles (4,600,000 rep) remains inactive.

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EXPLANATION OF FIGURES

FIGS. 1–28. Effect of the number of alpha particles striking the *Aspergillus niger* spore on the germination, growth, and sporulation of the fungus. The irradiated spores were incubated on diluted potato-dextrose agar, stained with Amann's lactophenol plus cotton blue and photographed at a magnification of ×300 except those with an asterisk (*) which are at ×350.

Fig.	*Dosage, per spore* *Polonium source*	*Alpha particles*	*Rep.* ×10⁻³	*Incubation time in hours*
1*	Nonirradiated Control[a]			6.5
2	1b	108	20	72
3*	1b	140	26	22
4*	4b	190	35	24
5*	Nonirradiated Control (in same desiccator as 4)			
6*	4b	280	52	20
7*	4a	290	53	144
8	1b	320	59	72
9*	4b	360	66	25
10	1a	398	73	72
11	1b	510	94	40
12	1a	510	94	40
13	3a	550	101	120
14	3b	600	110	90
15	2a	1170	215	43
16	2b	1170	215	43
17*	3a	1300	239	20
18*	2b	1400	258	72
19	2a	1620	298	25
20	2b	1800	331	44
21[a]	3b	2000	368	288
22[b]	1a	3900	718	48
23	1a	4950	911	47
24	1b	4950	911	47
25*	1a	5800	1067	43
26	1a	7000	1288	45
27*	4b	7700	1417	72
28	1a	8000	1472	43

[a] Unstained.
[b] Spores were irradiated in a drop of water.

THE GROWTH AND METABOLIC BEHAVIOR OF STREPTOMYCES VENEZUELAE IN LIQUID CULTURE

DAVID GOTTLIEB [1] AND MARVIN LEGATOR [2]

(WITH 3 FIGURES)

Physiological studies on the growth and metabolism of the actinomycetes have had an interesting history. Very soon after the discovery of these organisms the early investigators probed into the living processes of various species. In 1900, Beijerink (3) investigated pigment formation and was followed by Munter (23) who studied the nutrition of some members of this group. Krainsky's (21) detailed studies in chemically defined media are especially notable since they laid a foundation for many of the investigations which were to come. Through the years that followed, these microbes were almost entirely neglected except for the constant attention of Waksman who published a long series of papers on the metabolism of the actinomycetes (32, 33, 34, 35). This hiatus was almost uninterrupted until the advent of antibiotic therapy and the discovery of streptomycin (28). An interest in the activities of these organisms was reawakened but the researches were in the main confined to *Streptomyces griseus* (10, 11, 12, 15, 18, 19, 20, 27, 29, 30, 37). More recently the studies of Cochrane on *S. coelicolor* have established some of the biochemical systems present in this species (5, 6, 7, 8, 9). *S. venezuelae,* the subject of this paper, has also received some attention, but in this instance, too, it has been primarily in relation to antibiotic production (4, 13, 14, 16, 17, 24, 25, 31). Further studies of this interesting group of plants are important to determine how their processes fit into the general scheme of other

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living organisms. For this purpose the interrelationship of eight factors in growth and metabolism of *S. venezuelae* were studied; they are the morphological development of the organism, its rate of growth, and the changes in concentration of glycerol, lactic acid, total nitrogen, nitrate nitrogen, ammonia, and hydrogen ion.

METHODS

Streptomyces venezuelae strain 8–44 was used in these experiments. This organism can produce the antibiotic chloramphenicol in some media (**14, 16, 17**), but not in the medium used. This medium had the composition given below [3] and was prepared with chemicals of the C.P. or analytical grade, except for the sodium lactate which was available only in U.S.P grade. All observations and analyses were made in triplicate flasks. The medium was dispensed as 100 ml. aliquots in 500 ml. flasks and autoclaved for 20 min. at 15 lbs. pressure. Inoculum of *S. venezuelae* was prepared by growing the organism on modified Emerson agar (**16**) slants. After these cultures were incubated for 7 days at 28° C, 5 ml. of sterile distilled water were added to the slant and the spores rubbed off to make an aqueous suspension. Five ml. of this suspension were added to 100 ml. of liquid medium and the culture was grown for 48 hours on a reciprocal shaker. Two ml. of this culture were used to inoculate each of the flasks used in the experiment. Three flasks, taken at random, were removed from the shaker at various periods of time, from 0 to 192 hours. Aliquots from these flasks were used for all determinations. The morphological studies were made by microscopic observations of both wet mounts and Giemsa stained preparations. Weight of mycelium was obtained by centrifuging the liquid cultures in tared tubes, decanting the supernatant fluid, washing the centrifugate 3 times with distilled water, and finally drying the mycelium at 100° in a vacuum oven for 24 hours. The analytical procedures were adaptations of previously reported methods. Glycerol was

[3] Glycerol—10 gm.; KH_2PO_4—0.7 gm.; $K_2HPO_4 \cdot 3H_2O$—4.0 gm.; Na lactate—11.2 gm.; $NaNO_3$—0.7 gm.; NaCl—3.0 gm.; $MgSO_4$—2.0 gm.; $ZnSO_4 \cdot 7H_2O$—0.0115 gm.; $FeSO_4 \cdot 7H_2O$—0.0111 gm.; $CuSO_4$—0.0064 gm.; $MnCl_2 \cdot 4H_2O$—0.0079 gm.

determined by the colorimetric method of Mikkelson (22) and lactic acid by the method of Barker and Summerson (2). The standard microkjeldahl processes were used for total nitrogen except that the samples were predigested for 16 hours in a steam bath because the samples tended to foam badly during ordinary digestion (1). The analysis for ammonia in the culture fluid was the official method of the A.O.A.C. (1). Nitrate nitrogen was determined by the procedures described by Prince (26). Changes in hydrogen ion concentration of the medium were followed with a glass electrode pH meter.

RESULTS

The growth of *S. venezuelae* began slowly though not with the marked lag which has been described for bacteria (FIG. 2). This phase varied from 12 to 24 hours in the different experiments and was followed for the next 24 to 36 hours by a period of rapid growth which resembled the logarithmic increase of bacterial cultures. Growth rate was then reduced and the total amount of mycelium reached a maximum at about 72 hours. From then on, the total weight slowly decreased and tended to remain constant at about 60 to 70 per cent of the greatest weight. Morphological transformations in the culture corresponded in general to those which have been described for *S. griseus* (18). The spores of *S. venezuelae* germinated within the first 12 hours (FIG. 1 B), and mycelial fragments in the inoculum began visible growth at about the same time. The germ tubes of the spores then elongated into hyphae and side branches formed. This process continued and was accompanied by an anastomosing of the hyphae. At 24 to 36 hours the culture consisted of a large number of wefts of intermingled and anastomosed hyphae, more dense at the center than the edges. New centers of growth also formed from fragments of hyphae which broke off the weft during the agitation. No spores were observed during this period. With increased time of shaking, the size of the mycelial bundles increased; their centers became almost opaque and were surrounded by an open weft which terminated peripherally in young actively growing hyphal tips. Spore formation began at about 48 to 60 hours, and at 72 hours, when the weight of the mycelium was greatest, the culture contained numer-

ous spore chains. In shake culture, no clear differentiation existed between the sporophores and vegetative hyphae of young cultures. When broken from the mycelium the spore chains consisted of from one to many cells.

In these experiments, glycerol was the main source of carbon for the growth of the actinomycete; as the glycerol content of the medium decreased, the mycelium weight increased (FIG. 2). However, the medium was never entirely depleted of this component, and after 72 hours its concentration remained constant. This was the time at which the maximum weight of mycelium occurred and after which lysis began. Under such conditions further utilization of this alcohol would not be reasonably expected.

Lactic acid was not readily dissimilated by *S. venezuelae* although its concentration initially was equal to that of glycerol. Only 20 per cent of the lactic acid was removed from the medium. Growth occurred just as well in the absence as in the presence of this carbon source. That the poor utilization of the lactate was not due to the "sparing effect" of the glycerol was evident from the fact that even as alcohol was gradually depleted the rate of lactate utilization did not increase. No more lactate was consumed when the consumption of glycerol had stopped (FIG. 2). One can assume that the uptake of this component was dependent on the uptake of a more rapidly available carbon source.

The inorganic nitrogen was rapidly removed from the medium during the growth of *S. venezuelae* (FIG. 3). Eighty-six per cent of the nitrate nitrogen disappeared within the first 12 hours and 93 per cent within 48 hours; from then on the concentration of this component remained constant. When total soluble nitrogen was measured, the decrease in concentration in the medium paralleled the decrease of the nitrate ion for the first 60 hours. After 72 hours, the concentration of total soluble nitrogen increased slowly until 144 hours. The drop in total nitrogen in the early phases of growth reflects the removal of the nitrate, while the later increase indicates a lysis of some of the mycelium. This viewpoint is supported by the fact that growth and the total weight of the organism were beginning to decrease or had already ceased during the time of the increase in the total nitrogen concentration. Furthermore,

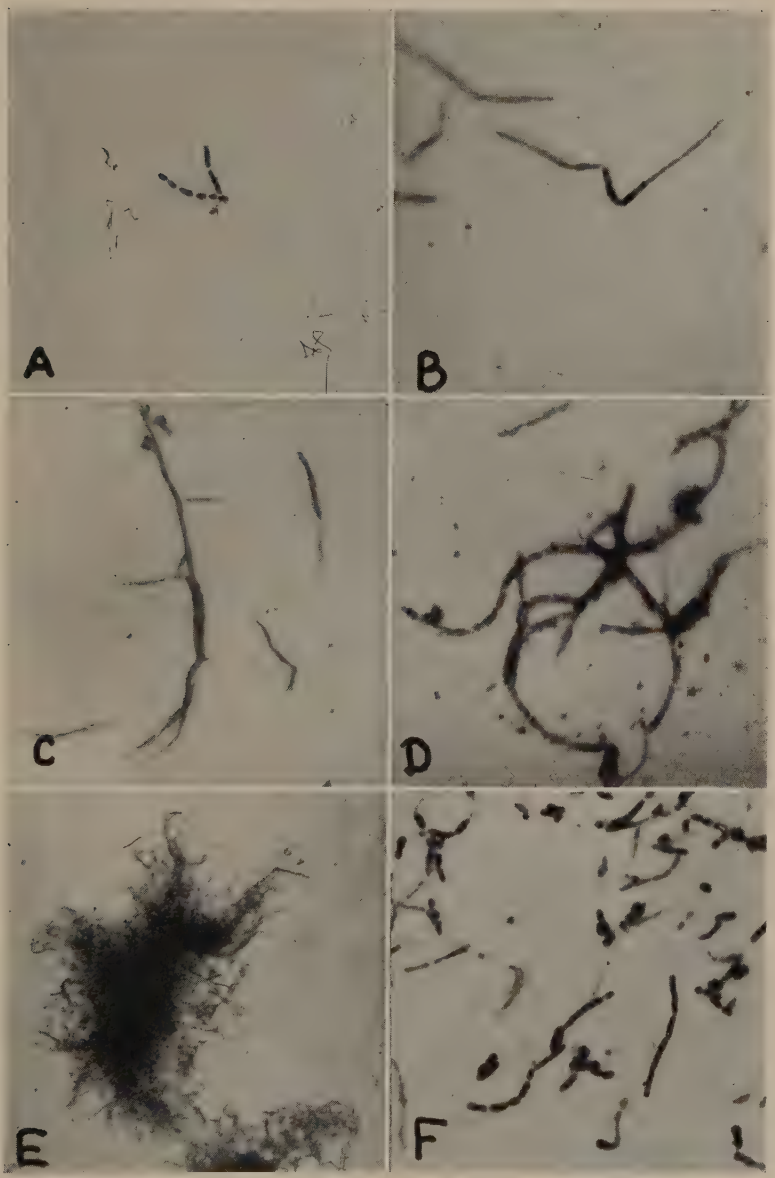

FIG. 1. Morphological changes of *S. venezuelae* in shake culture; A. Spores from inoculum, 12 hr. culture (× 970); B. Germinated spores in 12 hr. culture (× 970); C. Young hyphae 24 hr. culture (× 970); D. Developing mycelium in a 24 hr. culture (× 970); E. Mature mycelium in 48 hr. culture (× 100); F. Spores in 120 hr. culture (× 970).

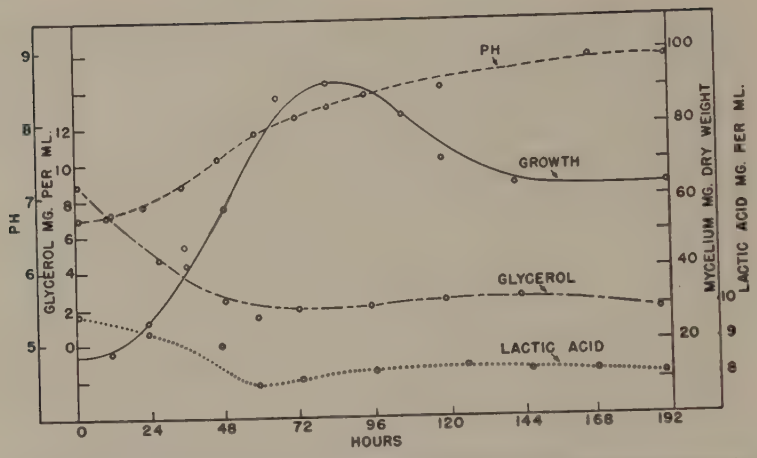

FIG. 2. The relationship between growth of *Streptomyces venezuelae* hydrogen ion concentration, the utilization of glycerol and sodium lactate.

the degradation of the mycelium can be observed microscopically during this period.

Ammonia is apparently a metabolic product during the assimilatory growth period. After a brief lag, which had about the same length as that for growth, the production of this nitrogen com-

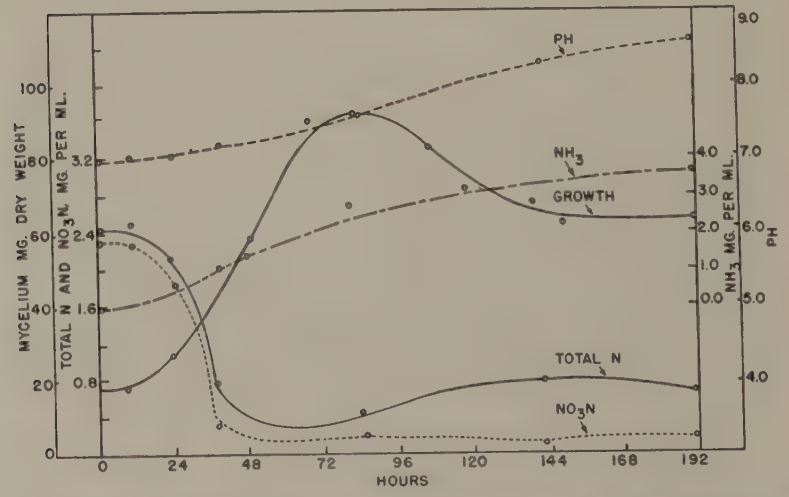

FIG. 3. The changes in various nitrogen components of the medium during the growth of *S. venezuelae*.

pound increased steadily during the first 60 to 72 hours and then the rate of production slowed and finally remained constant.

The hydrogen ion concentration of the medium rose steadily. However, the accumulation of hydroxyl ions can be ascribed to the production of ammonia since the curves for both components are parallel (FIG. 3). With glycerol as the carbon source, free acids are apparently not an important metabolic product.

SUMMARY

The "overall picture" of the growth, the morphological development and the uptake of the various components of a chemically defined medium during the culturing of *Streptomyces venezuelae* is for the most part straightforward. Spores germinate and give rise to hyphae which in turn form a loose mycelial weft. Later the weft changes to a more compact bundle with a dense center surrounded by young growing hyphae. The dry weight of the culture increases during this period; the carbon source, glycerol, disappears at about the time growth ceases. Lactic acid is utilized slightly and only in the period of glycerol utilization. During this time the medium is rapidly depleted of the nitrate ions, and ammonia is released, thereby increasing the hydroxyl ion concentration. Since the nitrate disappears long before the glycerol and before the weight of mycelium reaches its maximum, its assimilation into cellular material must be limited by the relatively low rate of absorption of the carbon source. After growth has reached its maximum, sporulation occurs and the mycelium tends to disintegrate. At this time the total soluble nitrogen in the medium increases because of the lysis of some mycelium.

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THE UTILIZATION OF CARBON AND NITROGEN COMPOUNDS BY USTILAGO ZEAE

FREDERICK T. WOLF

Although *Ustilago zeae* (Beckm.) Unger, the pathogen responsible for the disease known as corn smut, has been cultivated on synthetic media by a number of investigators, information is still incomplete concerning the range of carbon and nitrogen sources which this organism can utilize for growth. In many nutritional studies of fungi, only a single strain has been investigated. The present work is concerned with four monosporidial haploid cultures, representing all the meiotic products obtained from germination of a single chlamydospore. By this means, the role of various substances in the nutrition of several strains of the fungus may be ascertained. Further, possible variations in nutritional characteristics which might originate by meiosis would be revealed.

Maire (3) grew *U. zeae* successfully in Raulin's solution, and thus was one of the first workers to cultivate this fungus upon a synthetic medium. Ranker (5) studied the growth of nine strains of this organism in 26 different media, in attempts to devise a synthetic medium with optimal capacity for growth. A medium containing K_2SO_4, 0.3 gm.; NH_4NO_3, 0.1 gm.; $CaCl_2$, 0.1 gm.; $Mg_3(PO_4)_2 \cdot 4H_2O$, 0.1 gm.; and glucose 10 gm. per liter was found to be superior to all other combinations tested.

Volkonsky (8) studied the utilization of carbohydrates by *U. zeae,* finding that acid was produced from glycerol, xylose, glucose, levulose, galactose, maltose, sucrose, raffinose, and dextrin, but not from arabinose, rhamnose, lactose, or soluble starch. Schopfer and Blumer (6), in a study of the vitamin requirements of a number of species of *Ustilago,* established that no growth factors are required by *U. zeae.* Perkins (4) confirmed the finding that *U. zeae* was autotrophic with respect to growth factors, and by

ultra-violet irradiation produced a number of mutants heterotrophic for vitamins, amino acids, or nucleic acid components.

MATERIALS AND METHODS

The four monosporidial cultures of *U. zeae* were obtained from Dr. Russell Stevens, University of Tennessee. They were originally isolated with the aid of a micromanipulator, by Dr. Coyt Wilson, Alabama Polytechnic Institute, from the four sporidia of a single promycelium, from a smut gall collected at Auburn, Alabama. The cultures are designated A, B, C and D.

Cultures were grown in 125 cc Erlenmeyer flasks containing 50 cc of nutrient solution. In the experiments in which the carbon source was varied, the basal medium contained $NaNO_3$, 3.0 gm.; KH_2PO_4, 1.0 gm.; $MgSO_4 \cdot 7H_2O$, 0.5 gm.; KCl, 0.5 gm.; and 5 drops of 2% $FeCl_3$ per liter. The carbon sources tested, most of which were obtained from the Pfansteihl Chemical Company, Waukegan, Illinois, were added to the basal medium in quantities providing 400 mg. of carbon per 100 cc, and the pH was adjusted to 6.0.

In the experiments with compounds containing nitrogen, the basal medium contained KH_2PO_4, 1.0 gm.; $MgSO_4 \cdot 7H_2O$, 0.5 gm.; KCl, 0.5 gm.; 5 drops of 2% $FeCl_3$; and sucrose, 30 gm. per liter. The amino acids used were obtained from the Pfansteihl Chemical Company, Waukegan, Illinois; General Biochemicals, Inc., Chagrin Falls, Ohio; and Merck and Company, Rahway, New Jersey. For the inorganic nitrogen sources, glycine, β-alanine, and the *L*-amino acids, the nitrogen level was set at 25 mg. nitrogen per 100 cc. In the case of the *DL*-amino acids, the nitrogen level was increased to 50 mg. nitrogen per 100 cc, on the assumption that the *D*-isomer was unavailable to the fungus. The pH was adjusted to 6.0 in all instances.

Inoculations were made by adding 0.1 cc of a seven day old culture grown in Czapek's solution to each flask, by means of sterile pipettes. Growth was recorded after incubation for ten days at room temperature.

Considerable difficulty was experienced in establishing a satisfactory criterion of growth. This difficulty with *U. zeae* has been

discussed by Ranker (5), who found that removal of the cells from the medium by filtration, in an effort to determine weights, was impossibly slow. For this reason Ranker removed the cells by centrifugation, and employed their volume as a growth index. In the present work, filtration was also found to be unsatisfactory. Likewise, turbidity measurements with a Klett-Summerson photoelectric colorimeter were unreliable, because of the mycelial character of certain strains, resulting in the formation of clumps of cells. Growth was therefore estimated subjectively, and recorded on an arbitrary scale of 0, +, ++, +++, and ++++.

RESULTS

Twenty different carbohydrates were tested as sole sources of carbon for growth of each of the four strains of *U. zeae*. The results are presented in TABLE I:

It was found that glucose, levulose, mannose, sucrose, maltose and trehalose were the best of the carbon sources tested, and re-

TABLE I

THE GROWTH OF FOUR STRAINS OF *Ustilago zeae* IN MEDIA CONTAINING VARIOUS SOURCES OF CARBON

Carbon source	Strain			
	A	B	C	C
Glycerol	++	++	++	++
D-Arabinose	0	0	0	0
L-Arabinose	+++	+++	+++	+++
Xylose	+++	+++	+++	+++
Rhamnose	0	0	0	0
Glucose	++++	++++	++++	++++
Levulose	++++	++++	++++	++++
Mannose	++++	++++	++++	++++
Galactose	++	++	++	++
Sorbose	0	0	0	0
Sucrose	++++	++++	++++	++++
Maltose	++++	++++	++++	++++
Trehalose	++++	++++	++++	++++
Lactose	+++	+++	+++	+++
Cellobiose	++	++	++	++
Melibiose	0	0	0	0
Raffinose	+++	+++	+++	+++
Melezitose	+++	+++	+++	+++
Dextrin	+++	+++	+++	+++
Soluble Starch	0	0	0	0
Control (no carbon)	0	0	0	0

TABLE II

THE GROWTH OF FOUR STRAINS OF *Ustilago zeae* IN MEDIA CONTAINING VARIOUS SOURCES OF NITROGEN

Nitrogen source	Strain			
	A	B	C	D
$NaNO_3$	+++	+++	++++	++++
$(NH_4)_2SO_4$	++++	++++	++++	+++
Glycine	+++	+++	++	+++
Beta-Alanine	+++	+++	++	+++
L-Aspartic Acid	++++	++++	++++	++++
L-Glutamic Acid	+++	+++	+++	+++
L-Asparagine	++++	+++	+++	++++
L-Cystine	0	0	0	0
L-Cysteine.HCl	+	+	+	+
L-Leucine	+++	+++	+++	+++
L-Lysine.HCl	+++	++	++	++
L-Histidine.HCl	++	++	++	++
L-Arginine.HCl	+++	+++	+++	+++
L-Tryptophane	++	++	++	++
L-Tyrosine	+	+	+	+
L-Proline	+++	+++	+++	+++
L-Hydroxyproline	+	++	+	+
DL-Alanine	+++	+++	+++	++
DL-Serine	++++	++++	++++	++++
DL-Threonine	+++	+++	+++	+++
DL-Valine	+++	+++	+++	+++
DL-Isoleucine	+++	+++	+++	+++
DL-Norleucine	+++	+++	+++	+++
DL-Phenylalanine	+++	+++	+++	+++
DL-Methionine	+++	+++	++	+++
Control (no nitrogen)	0	0	0	0

sulted in maximal amounts of growth. *D*-arabinose, rhamnose, sorbose, melibiose and soluble starch were not utilized. Glycerol, *L*-arabinose, xylose, galactose, lactose, cellobiose, raffinose, melezitose and dextrin were utilized for growth, but the amount of growth produced was not as great as that produced in media containing the hexoses and disaccharides enumerated above. No variation among the four strains in ability to utilize carbohydrates was noted.

Sodium nitrate, ammonium sulphate and 23 different amino acids were compared as sole sources of nitrogen for growth of the four strains of *U. zeae*. The results of this experiment are presented in TABLE II:

Ammonium nitrogen appeared to be as good as nitrate under the conditions of these experiments. Almost all of the amino acids

tested were good sources of nitrogen for *U. zeae,* although no growth occurred in media containing cystine, and very little in media containing cysteine hydrochloride, tyrosine, or hydroxyproline. Aspartic acid, asparagine and serine were the best of the amino acids tested, and gave maximal amounts of growth, comparable to those obtained in ammonium sulphate. Some quantitative differences in amino acid utilization were noted among the four strains tested, but no differences of a qualitative nature were found.

DISCUSSION

When the present findings concerning carbohydrate utilization are compared with those of Volkonsky (8) agreement is obtained in all instances except with lactose. It will be recalled that Volkonsky employed acid production rather than growth as a criterion of utilization, which may explain the apparent discrepancy.

De Vay, Rowell and Stakman (1) have determined by paper chromatography that extracts of cultures of several strains of *U. zeae* definitely contain ribose, levulose, sucrose and maltose. In addition, arabinose, xylose, rhamnose and cellobiose were less certainly identified. All of these sugars have been shown to be utilized in the present experiments, except for ribose, which was not tested, and rhamnose, which was found not to be utilized. The determination of the sugars present in fungi by means of paper chromatography is deserving of further study in an effort to determine the fate of a single carbohydrate provided to the organism, and to determine the degree of correlation which might be established between results obtained with this technique and those from growth experiments.

Comparison of the present findings may also be made with those of Schopfer and Blumer (7) with *U. violacea,* the smut species which has hitherto been most thoroughly studied with respect to its carbon and nitrogen requirements.

The failure of *U. zeae* to attack starch would appear to be of particular interest, in view of the large quantities of starch present in the host plant, and also in view of the good growth obtained in dextrin, maltose, and glucose, which result from starch hydrolysis. The present findings with respect to starch, however, are

confirmatory of those of Grüss (2) and Volkonsky (8). The good growth on xylose is also of interest because of the large xylan content of the host.

No previous information concerning the utilization of amino acids by *U. zeae* has come to the attention of the writer. De Vay *et al.* (1) by paper chromatography have demonstrated the presence of 15 different free amino acids in cells of this species. The present experiments indicate the tryptophane is a rather poor source of nitrogen for growth of *U. zeae*. Tryptophane, however, has been shown to be the precursor of indole acetic acid, which is involved in the pathological changes in plants with corn smut disease (Wolf, 9).

On the assumption that the utilization of various carbohydrates and amino acids are characters which are inherited and thus are genetically controlled, it might have been expected that segregation would have occurred at meiosis. No qualitative differences in utilization of any of the 20 carbon sources or 25 nitrogen sources were observed, however, among the four haploid strains resulting from meiosis. No information is available concerning the nutritional characteristics of the two strains which presumably mated to produce the chlamydospore from which the four strains studied were derived. It is possible that the original chlamydospore was homozygous for all characters studied. These experiments, therefore, yield little information concerning the mode of inheritance of nutritional characters. The occurrence of quantitative differences in utilization of some of the amino acids among the four strains would suggest the possibility that multiple factors may be involved in the utilization of a single substrate.

The writer is pleased to acknowledge the assistance of Mr. William T. Allman, Jr., which was made possible by a fellowship from the General Education Board.

SUMMARY

This study is concerned with the growth of *Ustilago zeae* in synthetic media containing various sources of carbon and nitrogen. Four monosporial cultures of *U. zeae*, all derived from a single promycelium, were studied comparatively. Glucose, levulose,

mannose, sucrose, maltose and trehalose were the best of 20 carbon sources tested. *D*-arabinose, rhamnose, sorbose, melibiose and starch were not utilized. *U. zeae* is able to utilize nitrate, ammonium, or amino nitrogen. Good growth was obtained in 19 of 23 amino acids tested as sole sources of nitrogen, aspartic acid, asparagine, and serine proving to be superior to other amino acids. Little or no growth was obtained with cystine, cysteine hydrochloride, tyrosine, or hydroxyproline. No qualitative differences in utilization of any of these substances were noted among the four strains of *U. zeae* studied.

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COMPARISONS OF THE SURVIVALS OF ASPERGILLUS NIGER SPORES LYOPHILIZED BY VARIOUS METHODS [1]

R. H. Haskins and Joan Anastasiou [2]

(with 1 figure)

Though the fundamentals of freeze-drying were known (but not fully appreciated) at least as early as 1813 (12), the first clearly recorded use of sublimation for preserving biological substances was that of Shackell in 1909 (8). The preservation of bacteria, viruses, and yeasts by the lyophil process has received considerable attention since then, but only in the last decade have attempts to preserve mold cultures by lyophilization been successful. In this respect the work of Raper and Alexander (7) is outstanding.

It has been reported (11) that special characteristics may be lost from bacterial cultures during lyophilization. Certain fungi do not seem to be able to survive the lyophil process. Others, while they survive apparently unchanged morphologically, do not retain all of their original characteristics—creating serious problems for the industrial fermentations workers.

It was considered important, therefore, to investigate, methodically, the factors affecting the survival of lyophilized fungous spores, to determine the crucial steps in the various procedures, and to attempt to develop new procedures or to so modify old procedures as to make possible the preservation of all important fungi with retention of all their original characteristics.

[1] Paper presented, in part, before a joint session of the Mycological Society of America and the Microbiological Section of the Botanical Society of America at the Cornell University Meetings of the A.I.B.S., September 8–10, 1952. Issued as Paper No. 153 on the Uses of Plant Products and as N.R.C. No. 3003.

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A number of such studies have been made on bacteria (e.g. Wolff, 11) while others (e.g. 4, 5, 10) are in progress on fungi. This paper reports the results of several preliminary experiments with the fungus *Aspergillus niger*. The most efficient of the three techniques now in common use for the lyophil preservation of fungous cultures was determined, and modifications of techniques involving pre-lyophilization treatments and various suspending vehicles were examined.

METHODS

Three methods of preservation of fungous spores in the lyophil state were compared in these preliminary studies. The first, for convenience designated the NRRL method, is essentially that of Raper and Alexander (7). It involves snap-freezing a suspension of spores in a suitable vehicle, evacuating the tube containing the suspension, first at low temperatures, then eventually at room temperature, and finally sealing off the tubes containing the dried spore suspensions *in vacuo*. During the process the vacuum is not broken.

The second method, for convenience designated the PRL method, is similar to the NRRL method except that the tubes containing the spore suspensions are frozen in a freezer-chest, primary drying is carried out in a vacuum desiccator (3) or other suitable container at room temperatures, and secondary drying and sealing off are done on a suitable manifold arrangement. In this process, the vacuum is broken between primary and secondary drying.

The third method is a centrifugal freeze-drying technique, based on work by Greaves (2), in which the tubes containing the unfrozen spore suspensions are angle-centrifuged during the initial stages of evacuation to prevent boiling, and until evaporative (auto-) freezing has taken place. After primary drying, the vacuum is broken, and the tubes mounted on manifold where, following secondary drying, they are sealed off, leaving their contents *in vacuo*.

The vacuums used in each of the processes ranged from 100–50 μ mercury pressure during primary drying, and 50–25 μ mercury pressure during secondary drying and sealing off. Tempera-

tures for snap-freeze ranged from -35 to $-65°$ C, and for primary drying according to the NRRL method about $-10°$ C. The vapor trap was maintained at temperatures from -45 to $-65°$ C.

Throughout the work, *Aspergillus niger* PRL *24* was the test organism, and was harvested from the sporulation medium of Gastrock *et al.* (1). The harvesting of spores and the preparation and filling of the tubes were done in the usual manner and procedures standardized as much as possible. Similarly the techniques of opening of the tubes and revival of preserved organisms were standardized and controls included in each test. The percentage survival of spores was determined by direct count of germinated and ungerminated spores following standardized incubation conditions. At least 1000 spores were counted from each lyophil tube tested, with counts from 5–8 tubes included in each average value.

APPARATUS

For the NRRL and PRL techniques and for secondary drying and sealing-off of the centrifuge technique, a small but convenient table apparatus was designed. This apparatus, shown in FIG. 1, consisted of a drying chamber (DC) for primary drying according to the PRL method; a manifold (M) for the whole of the NRRL technique, and the secondary drying and sealing off of both the PRL and centrifuge techniques. A vapor trap (VT) is provided for immersion in a dry-ice, ethylene glycol and water freezing mixture and is so constructed as to be easily and quickly replaced should it become plugged with sublimed water. A large capacity vacuum pump is attached to the central tube (VP) of the vapor trap. A vacuum release (VR) and McLeod vacuum gage (VG) are provided as shown. For more efficient drying the connections between the material to be dried and the vapor trap were made as short and large as practicable.

For the primary drying during the centrifuge technique, an Edwards Model 3PS Centrifuge Freeze-drier [3] was used. This is

[3] Made by W. Edwards & Co. (London) Ltd., Lower Sydenham, London S.E. 26, England. Canadian distributors: Scientific Exports (Great Britain) Ltd., 50 York Street, Toronto 2, Canada.

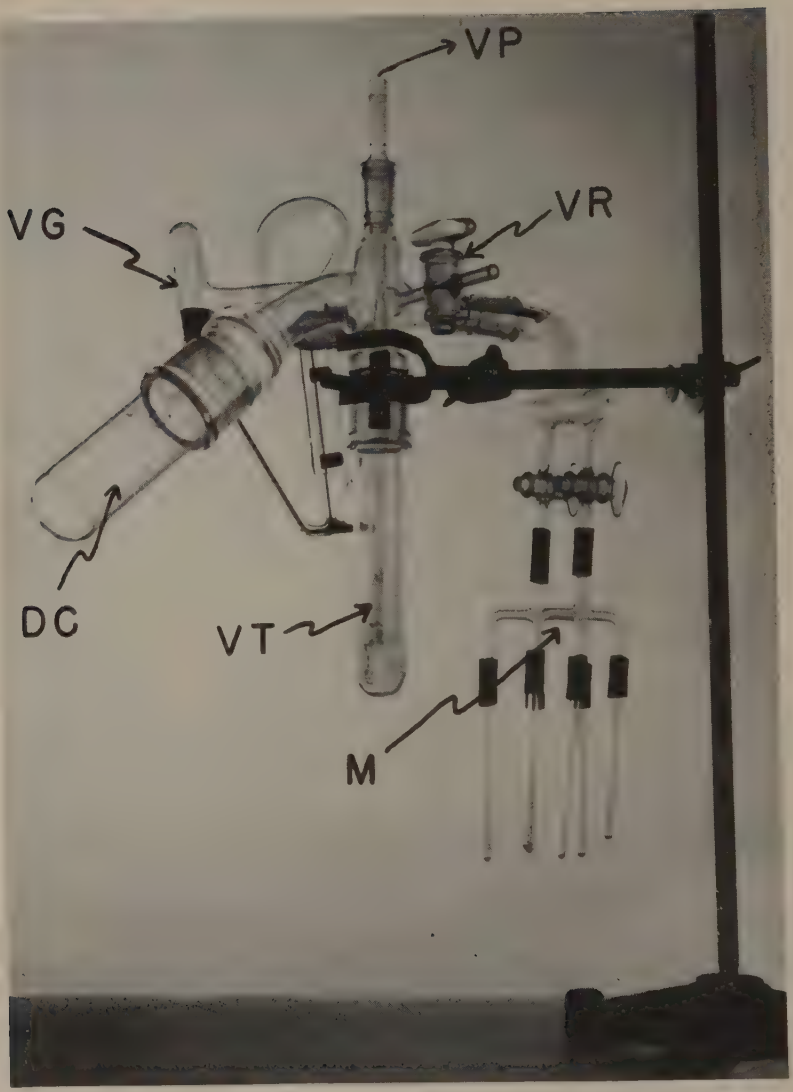

FIG. 1. Table lyophil apparatus. For explanation see text.

a self-contained unit capable of handling, simultaneously, 400 lyophil tubes primary drying, and 400 tubes secondary drying. In our studies, for uniformity, all secondary drying was done on the table apparatus regardless of the lyophil technique being used.

EXPERIMENTAL AND RESULTS

At first, the survival of *A. niger* spores lyophilized in beef serum by the NRRL and by the PRL techniques were compared. The results are shown in TABLE I (a). Controls were treated the same as other tubes except they were not lyophilized, of course, and germination was tested immediately. The table shows that, while the PRL technique gave slightly better results, there was no significant difference in results from the two methods.

Next, both the NRRL and PRL techniques were compared with the centrifuge method. It is obvious from TABLE I (b) and (c) that a significantly greater percentage germination was obtained with the centrifuge technique than with either the NRRL or the PRL techniques.

In 1949 Weston (**10**) reported higher percentage germination of lyophilized fungous spores when the spore suspensions were

TABLE I

COMPARISONS OF THE SURVIVAL OF *A. niger* SPORES LYOPHILIZED IN BEEF SERUM BY VARIOUS TECHNIQUES

Technique	No. of runs	Per cent germination[1]	
		Average	Range
(a)			
NRRL	7	30.7	10–77
PRL	7	34.1	15–78
Controls	7	93.4	83–98
(b)			
NRRL	5	32.1	16–58
Centrifuge	5	54.3	44–61
Controls	5	95.4	92–98
(c)			
PRL	7	53.8	26–75
Centrifuge	7	67.8	47–87
Controls	5	84.4	72–98

[1] NRRL and PRL techniques did not differ significantly. Differences were significant between NRRL and centrifuge techniques ($P < 0.05$) and between PRL and centrifuge techniques ($P < 0.05$).

TABLE II

THE EFFECT OF WETTING AGENTS AND PRE-LYOPHILIZATION TREATMENT ON THE SURVIVAL OF *A. niger* SPORES LYOPHILIZED IN BEEF SERUM BY VARIOUS TECHNIQUES

Treatment		Per cent germination[1]					
		NRRL	PRL	Control	Centrifuge		Control
					Average	Range	
without wetting agent	without pre-evacuation	11.8	23.1	91.7	51.3	35–62	
	with pre-evacuation	22.9	45.2		56.9	49–68	86–89
with wetting agent	without pre-evacuation	21.8	20.4	97.2	45.1	30–51	87–88
	with pre-evacuation	34.5	42.1		59.5	46–65	
wetting agent		"Vel"	"Dreft"		"Dreft"		
number of runs		1	1		6		

[1] Analysis of the results for the centrifuge technique only: no significant difference between "with wetting agent" and "without wetting agent" but the difference between "with pre-evacuation" and "without pre-evacuation" was significant ($P < 0.01$).

subjected to dehydration by preliminary evaporation in the liquid state followed by final sublimation from the frozen state. In the next experiment were studied briefly the effects of such pre-lyophilization treatment and of treatment with wetting agents on the survival of *A. niger* spores lyophilized in beef serum by each of the three techniques under consideration. Some of the runs had wetting agent added to the suspending vehicles, others had none added. Both "Vel" and "Dreft" were used as wetting agents. Only sufficient "Vel" or "Dreft" was added to produce a slight foam when the suspension was shaken. This appeared to be enough to wet the spores. In the amounts used, "Vel" and "Dreft" were found to have similarly negligible effects on spore germination. Half of the runs were subjected to reduced pressure at room temperature for 20–30 minutes immediately prior to lyophilization. The pressure was reduced, by means of an aspirator pump, almost to the foaming point of the suspension.

With the NRRL technique (TABLE II) both the use of wetting agents and preliminary evacuation markedly increased per cent germination. With the PRL technique the use of a wetting agent was not beneficial, but preliminary evacuation permitted a 50% increase in germination. With the centrifuge technique, the use of a wetting agent showed no beneficial effect, but preliminary evacuation resulted in significantly increased germination.

This confirms, as first shown by Weston (**10**), that a preliminary evaporation from the liquid state before freezing and drying does result in an increase in per cent germination. This increase does not seem to be due to more efficient wetting of the spore surface, since it was found that letting the spore suspensions stand after preliminary evaporation before lyophilization lessens the effect, indicating perhaps that the beneficial effects of the treatment may be due to a degassing of the spore suspension. There was no such change in the effect of wetting agent when the wetting agents containing suspensions were permitted to stand before processing.

Since the centrifuge technique had proved the most effective of the three methods, it was used in a comparison of various suspending vehicles. The vehicles used and results obtained are shown in TABLE III. Though skim milk has been used extensively as a

TABLE III

A COMPARISON OF THE SURVIVAL OF *A. niger* SPORES LYOPHILIZED IN VARIOUS CARRIERS, USING THE CENTRIFUGE TECHNIQUE

Carrier	Number of runs	Total No. tubes counted	Per cent germination	
			Average	Range
"Kelgin"[1] (0.1%)	3	6	36	15–52
Water	4	9	37	27–56
Beef serum	3	6	43	39–49
Casein hydrolyzate (vitamin free)	4	9	47	15–63
Peptone (10%)	4	6	66	25–76
Sucrose (20%) + ascorbic acid (0.5%)	2	2	71	70–72
Glucose (20%)	2	2	81	78–85
Sucrose (20%) + peptone (10%)	3	3	81	57–99
Sucrose (20%)	6	9	85	70–97

[1] An algin provided by Kelco Company, Los Angeles, California.

suspending vehicle in these laboratories, it could not be included in this comparison, because the opaqueness of material precluded the possibility of determination of per cent germination by direct count.

Of the vehicles reported on here, glucose and sucrose give the best results, confirming the work of Weston (**10**). The use of glucose, sucrose, etc. as vehicles does not result in formation of loose, dried pellets that are so easy to remove from the lyophil tubes when one wishes to revive a lyophilized culture. The use of the centrifuge technique has the same disadvantage, but the increase in per cent germination using such vehicles and such a technique may be sufficient to more than offset this disadvantage, particularly if the methods prove to be more capable of preserving specific physiological characteristics than the methods now in common use.

Though suspending vehicles appear to be emphasized here, their use is not always advisable. Sharp and Smith (**9**) have shown that certain rust uredospores are readily preserved by lyophilization without change in pathogenicity when no suspending vehicle is used, but have no measurable survival when lyophilized in the commonly used suspending media—blood serum, gelatin or sucrose solutions.

DISCUSSION

That fungous spores survive the centrifuge method of freeze-drying better than they survive the other two methods under consideration, is not surprising when the results of studies of the low temperature death of microorganisms (**4**) are considered. With the centrifuge method, while data on the temperature changes in the spore suspension during centrifugation, evacuation and drying are difficult to obtain, it is certain that these changes are in no way as drastic as those imposed during the NRRL and PRL methods.

Wolff (**11**) has shown for bacteria, and Mazur (**4**) for fungi, that the rate of thawing is critical particularly between about $-10°$ C and $-35°$ C. For bacteria, better survival resulted from drying at $-35°$ C than from freezing to $-35°$ C and later raising temperature to $-10°$ C during drying. With fungi, death occurred mostly in rigid frozen vehicles at temperatures between

$-15°$ C and $-35°$ C (4), particularly when thawing between these temperatures was slow.

While freezing in the NRRL and PRL methods, as described above, is rapid, raising of the temperatures of the frozen suspensions is carried out more slowly, perhaps accounting in part for the lower survival permitted by these methods.

Why a preliminary evaporation from the liquid state followed by sublimation from the frozen state should give a survival better than when the preliminary evaporation is omitted, is not yet understood. It does not appear to be a wetting phenomenon. Studies on the degassing effect of the treatment are continuing.

The problem of which suspending vehicle to use assumed considerable importance when it was discovered (10) that 20% sucrose gave much better survival with fungous spores than did the "protective colloids" so emphasized by workers studying preservation of bacteria (e.g. 6), and when it was shown (9) that the use of suspending vehicles is not always advisable.

It is probable that no single technique will be developed that will be equally suitable for the preservation of all microorganisms. The particular property or properties of the organism upon which selection of the lyophilization technique hinges have yet to be discovered.

SUMMARY

1. The percentage germination of *Aspergillus niger* spores following a centrifuge freeze-drying method was significantly higher than that following two other commonly used methods involving snap-freezing to low temperatures.

2. The higher per cent germination resulting from a preliminary evaporation in the liquid state followed by final sublimation from the frozen state did not appear to be due to more efficient wetting of the spore surface, but rather to a degassing effect.

3. Of the limited series of suspending vehicles tested with the centrifuge method, 20% glucose or sucrose permitted the greatest survival (over 80%) of the fungous spores.

4. The use of such vehicles and of the centrifuge method of preserving spores does not result in the formation of loose dried pellets which are easily removed from the tube when the culture

is to be revived, but the increase in survival may be sufficient to more than offset this disadvantage.

ACKNOWLEDGMENTS

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THE PRODUCTION AND ACTION OF A TARTRATE–DECOMPOSING ENZYME

Richard R. Barton

(with 4 figures)

At the present time, the principal method of eliminating argols (crude potassium bitartrate crystals) from grape products is to speed their crystallization and to remove the crystals by filtration. Despite the fact that many micro-organisms are characterized by their ability to utilize tartrates (Fulmer-Werkman, **5**; Stadtman, Vaughn and Marsh, **14**), there has been no effort to use them or their enzyme systems to prevent argol formation.

Several fungi were isolated by Powers (**11**) from wine lees and grape pomace piles that were capable of utilizing potassium bitartrate as their sole source of carbon. Of these, a strain of *Asperigillus versicolor* (Vuill.) Tiraboschi was the most effective in that it utilized 87.7 per cent of the tartrates in solution. In order to utilize this fungus for tartrase production, it was necessary to ascertain the cultural method best suited for enzyme production, to determine the ability of various strains of this fungus to produce an active enzyme, to determine the relation between metallic salts added to the substrate and enzyme activity, to ascertain the relationship between external factors and tartrase activity, and to identify the break-down product of the enzyme catalyzed reaction.

METHODS AND MATERIALS

Culture of Aspergillus versicolor and extraction of tartrase. Investigation showed that the medium most suitable for the culture of *A. versicolor* for tartrase production was ground grape pomace containing an added 1 per cent potassium bitratrate with the pH adjusted to 3.0. Fifty gram portions of this medium were spread on the bottom of 500 ml. wide mouth Erlenmeyer flasks and sterilized at 15 pounds pressure for 20 minutes. In order to obtain

rapid fungus growth on this medium, it was necessary to first introduce the fungus spores in a liquid medium containing 0.7 per cent yeast extract, 0.5 per cent potassium acid phosphate and 5 per cent dextrose in order to obtain germination. About 10 ml. of this medium containing the germinated spores were used to inoculate 50 grams of the solid medium. Heavy mycelial growth was obtained in three to seven days incubation at 30° C, depending on the strain of the fungus used.

For maximum tartrase activity, the enzyme was extracted from the fungus just before spore formation occurred. Fifty grams of the moldy medium were macerated and mixed with 500 ml. of cold (8° C) water and stirred for one hour to extract the tartrase. In order to remove particles of medium and mycelia, about 200 grams of filter aid were mixed with the extract before filtration through a Buchner funnel.

As with several other enzymes (Crewther and Lennox, **2**; Stark and Tetrault, **15**; and Tauber, **16**), tartrase was insoluble in ethyl alcohol. In order to precipitate the enzyme from the extract, two volumes of cold (8° C) 95 per cent ethyl alcohol were added to each volume of enzyme extract. The enzyme was separated from the supernatant liquid by means of a centrifuge. This moist enzyme preparation was used to ascertain the tartrase activity of the different strains of *A. versicolor* tested and to study the relation between metallic salts added to the substrate and tartrase activity. Since this moist preparation would lose its activity within 24 hours, it was necessary to dry it in order to obtain a stable preparation. Using an adaption of the apparatus of Flosdorf (**3**), the enzyme was dried by lyophilization.

Ability of different strains of Aspergillus versicolor to produce an active enzyme. The ability of different strains of fungi to utilize certain nutrient substances or to produce active enzymes varies over a wide range (Foster, **4**; Harter and Weimer, **7**; Menon, **10**; and Raper, Alexander and Coghill, **12**). Eight different strains of *A. versicolor* were obtained from different sources. From the strain which produced the most active tartrase, 91 single spore isolate cultures were obtained by means of a micromanipulator. In addition, several of these single spore isolate

cultures were treated with radio-active phosphorus in an effort to obtain mutants which would produce more active enzyme preparations.

To use a uniform amount of material from each culture, the enzyme from 50 ml. of aqueous extract was added to 100 ml. of a 1 per cent tartaric acid or potassium bitartrate substrate. After incubation of 30° C for 20 hours, the tartrase activity was ascertained by determining the per cent tartrate decomposition using the sodium metavanadate colorimetric determination of Underhill, Peterman and Kraus (**17**).

Since it was found that tartrase was an adaptive enzyme (Virtanen, **18**), it was necessary to culture the different strains of *A. versicolor* on slants that contained potassium bitartrate as a major source of carbon in order to retain their original tartrase activity.

Relationship between metallic salts added to the substrate and tartrase activity. This work was divided into two sections. In the first part the following concentrations of metallic salts were used: ferrous chloride, 0.1 per cent; zinc chloride, 0.1 per cent; cuprous chloride, 0.0005 per cent; ammonium molybdate, 0.01 per cent; magnesium sulfate, 0.25 per cent, and manganese sulfate, 0.02 per cent. Further work was done using a combination of ferrous chloride and other salts under test. The second part of this work was to determine the action of various concentrations of ferrous chloride and a combination of ferrous chloride and zinc sulfate. The ferrous chloride was used in concentrations ranging from 0.04 to 0.20 per cent in 0.02 per cent increments. The concentrations of ferrous chloride and zinc sulfate used were the same. In each case, however, the total salt concentration was maintained at 0.24 per cent.

The enzyme from 50 ml. of enzyme extract was added to 100 ml. of substrate containing the added metallic salts. After 20 hours incubation at 30° C, enzyme activity was determined. Three replicates of each determination were made.

Relation between external factors and tartrase activity. To ascertain the relationship between time, temperature and pH of the substrate and tartrase activity, 10 mg. of a dried tartrase prepara-

tion and 8 mg. of ferrous chloride were added to 10 ml. of a 1 per cent potassium bitartrate substrate. The same amounts of tartrase and ferrous chloride were added to 10 ml. aliquots of substrate containing varying concentrations of potassium bitartrate to determine the relationship between concentration of substrate and tartrase activity. Concentrations of tartrase ranging from 0.005 to 1.00 per cent were used in the study of the relationship between tartrase activity and concentration.

Identification of enzymatic breakdown products of tartaric acid. Total acid and pH measurements of the substrate before and after the enzymatic reaction had occurred indicated that there was very little change in either measurement and that there was no trend towards either greater acidity or alkalinity. Because of this it was assumed that the breakdown product was an acid. The filter paper chromatogram procedure of Lugg and Overell (9), was used to identify this acid.

RESULTS

Ability of strains of Aspergillus versicolor to produce tartrase. The eight strains of *A. versicolor* obtained from different sources produced tartrase preparations of varying activity (TABLE I). This activity ranged from 36.1 to 49.1 per cent tartrate decomposition when the tartrates were supplied in the form of tartaric acid.

The tartrase from 30 of the 91 single spore isolates obtained from the culture of *A. versicolor* obtained from Dr. W. D. Gray

TABLE I

ENZYMATIC DECOMPOSITION OF TARTARIC ACID BY ENZYMES EXTRACTED FROM STRAINS OF *Aspergillus versicolor*

Source and strain number	Initial tartrate concentration, mg./ml.	Final tartrate concentration, mg./ml.	Per cent decomposition[1]
O.A.E.S. *7A*	10.8	6.8	38.1
Gray (OSU) *67*	10.8	5.5	49.1
NRRL *226*	10.5	5.4	48.5
NRRL *227*	10.8	6.5	39.8
NRRL *231*	10.8	6.5	39.8
NRRL *233*	11.0	6.5	40.9
NRRL *235*	10.5	5.4	48.5
NRRL *238*	10.8	6.9	36.1

[1] Incubation time, 20 hours at 30° C.

TABLE II

TARTRASE ACTIVITY OF ENZYMES EXTRACTED FROM STRAINS OF *Aspergillus versicolor* GROWN ON MEDIA CONTAINING RADIO-ACTIVE PHOSPHORUS

Strain No.	Before irradiation	Tartrase activity[1]	
		Immediately after subculturing from irradiation medium	Six months after treatment
4	22.9	37.6	22.9
8	19.3	39.3	18.3
9	22.9	32.8	22.5
10	25.3	39.3	25.7
11	22.9	32.8	23.0
13	20.4	45.0	20.6
14	25.3	37.6	25.7
15	19.3	41.8	19.5

[1] Expressed as percent tartrate decomposition.

of the Botany Department of The Ohio State University was tested on a tartaric acid substrate. It was found that the activity of these preparations ranged from a low of 30.6 per cent decomposition to a high of 64.5 per cent decomposition. On analysis it was found that the mean decomposition was 46.6 per cent and the standard deviation was 9.26 per cent.

Since the argols in grape products consist largely of potassium bitartrate, the tartrase from 81 of the 91 single spore isolate cultures was tested on substrates containing this salt. Tartrase activity was considerably lower against potassium bitartrate than against tartaric acid, ranging from 1.3 to 34.2 per cent decomposition. The mean decomposition was 22.3 per cent and the standard deviation was 6.05 per cent.

From eight of the single spore isolate cultures treated with radioactive phosphorus, the tartrase was extracted and tested immediately after treatment and again six months later. As can be seen in TABLE II, immediately after treatment with this isotope, the enzymes were more active (an average of 16 per cent higher) but six months after treatment their activities had dropped back to what it was in the original single spore isolates.

Single spore isolate culture No. 76 produced the most active tartrase and was used as the source of this enzyme for the remainder of the work.

TABLE III

RELATION BETWEEN METALLIC SALTS ADDED TO SUBSTRATE AND TARTRASE ACTIVITY[1]

Salt	Concentration		Per cent potassium bitartrate decomposition[2]
	Gms./100 ml.	Molarity	
$ZnSO_4$	0.100	0.0062	29.0
$FeCl_2$	0.100	0.0079	37.5
$CuCl_2$	0.0005	0.000037	26.3
$(NH_4)_2MoO_4$	0.001	0.000055	22.5
$MgSO_4$	0.250	0.0206	23.5
$MnSO_4$	0.020	0.0013	20.5
Control	—	—	15.5

[1] Single spore culture No. *76* used.
[2] Average of three replications.
LSD 3.21 and 4.45 at 5% and 1% levels.

Activation of tartrase by addition of metallic salts to the substrate. When iron, zinc and copper salts were added to the substrate, tartrase activity was significantly higher at the 1 per cent level than that of the control (22.0 to 10.8 per cent higher). When molybdenum, magnesium and manganese salts were present enzyme activity was significantly higher at the 5 per cent level (TABLE III).

In the presence of a combination of iron and the other salts tartrase activity was higher in the presence of a combination of

TABLE IV

RELATION BETWEEN COMBINATION OF IRON AND OTHER METALLIC SALTS ADDED TO SUBSTRATE AND TARTRASE ACTIVITY[1]

$FeCl_2$ Gms./100 ml.	Molarity	Metallic salt	Concentration		Per cent potassium bitartrate decomposition[2]
			Gms./100 ml.	Molarity	
—	—	$FeSO_4$	0.100	0.0066	30.7
0.100	.0079	—	—	—	32.1
0.100	.0079	$ZnSO_4$	0.100	0.0062	36.8
0.100	.0079	$CuCl_2$	0.0005	0.000037	36.2
0.100	.0079	$(NH_4)_2MoO_4$	0.001	0.000055	33.6
0.100	.0079	$MgSO_4$	0.250	0.0206	39.5
0.100	.0079	$MnSO_4$	0.020	0.0013	37.5
Control		—	—	—	29.7

[1] Single spore culture No. *76* used.
[2] Average of three replications.
LSD 2.86 and 3.97 at 5% and 1% levels.

TABLE V

RELATION BETWEEN VARYING CONCENTRATIONS OF $FeCl_2$ AND $ZnSO_4$ ADDED TO SUBSTRATE AND TARTRASE ACTIVITY[1]

Salt concentration, gms./100 ml.		Per cent potassium bitartrate decomposition[2]
$FeCl_2$	$ZnSO_4$	
0.04	0.20	22.2
0.06	0.18	24.1
0.08	0.16	28.1
0.10	0.14	29.2
0.12	0.12	26.0
0.14	0.10	31.1
0.16	0.08	31.1
0.18	0.06	31.5
0.20	0.04	33.0
Control	—	18.1

[1] Single spore culture No. *76* used.
[2] Average of three replications.
LSD 1.45 and 1.99 at the 5% and 1% levels.

iron and zinc, iron and copper, iron and magnesium, and iron and manganese than in the presence of iron alone (TABLE IV).

When varying concentrations of iron and zinc were added to the substrate, tartrase activity increased from 22.2 per cent decomposition when 0.04 per cent iron and 0.20 per cent zinc were used to 33.0 per cent decomposition when 0.20 per cent iron and 0.04 per cent zinc were present (TABLE V). In the presence of varying iron concentrations, tartrase activity increased from 22.6 per

TABLE VI

RELATION BETWEEN VARYING CONCENTRATIONS OF $FeCl_2$ ADDED TO SUBSTRATE AND TARTRASE ACTIVITY[1]

Concentration of FeCl, gms./100 ml.	Per cent potassium bitartrate decomposition[2]
0.04	22.6
0.06	23.5
0.08	26.6
0.10	28.5
0.12	27.3
0.14	30.1
0.16	28.5
0.18	31.6
0.20	31.6
Control	21.5

[1] Single spore culture No. *76* used.
[2] Average of three replications.
LSD 3.08 and 4.15 at 5% and 1% levels.

cent decomposition to 31.6 per cent decomposition as the iron concentration increased from 0.04 to 0.20 per cent (TABLE VI).

Relation between external factors and tartrase activity. In this work, in so far as possible, all factors were kept constant except the one under study.

Tartrase activity was determined at time intervals of 2, 4, 6, 8 and 26 hours incubation at 40° C. The potassium bitartrate concentrations at the beginning and the end of each time interval were

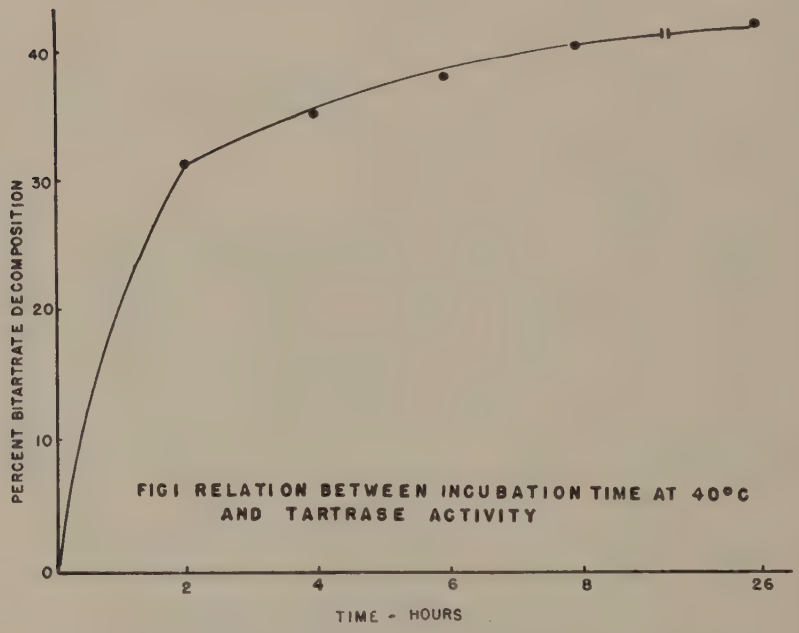

FIG. 1. RELATION BETWEEN INCUBATION TIME AT 40°C AND TARTRASE ACTIVITY

calculated as molar concentrations in order to compute the velocity constants of the enzyme catalyzed reaction. These constants were calculated using the first order reaction equation. As can be seen in FIG. 1, 78.8 per cent of the total enzyme activity occurred in the first two hours of incubation and then increased slowly. The velocity constants decreased from a high of 0.0882 at two hours incubation to a low of 0.008 at 26 hours incubation. It can also be seen that at the end of eight hours incubation 96.0 per cent of the total tartrate decomposition had occurred.

In this study substrate-enzyme mixtures were incubated for two hours at 20° C, 30° C, 40° C, 50° C, and 60° C. From FIG. 2 it can be seen that the optimum temperature for this enzyme catalyzed reaction was 50°C. The velocity constants which were calculated with the same equation used above increased as the temperature increased until a maximum of 0.0994 was reached at 50° C and then decreased as the temperature increased further. At 50° C the tartrase activity was 36.8 per cent decomposition which was significantly higher than at 40° C or 60° C.

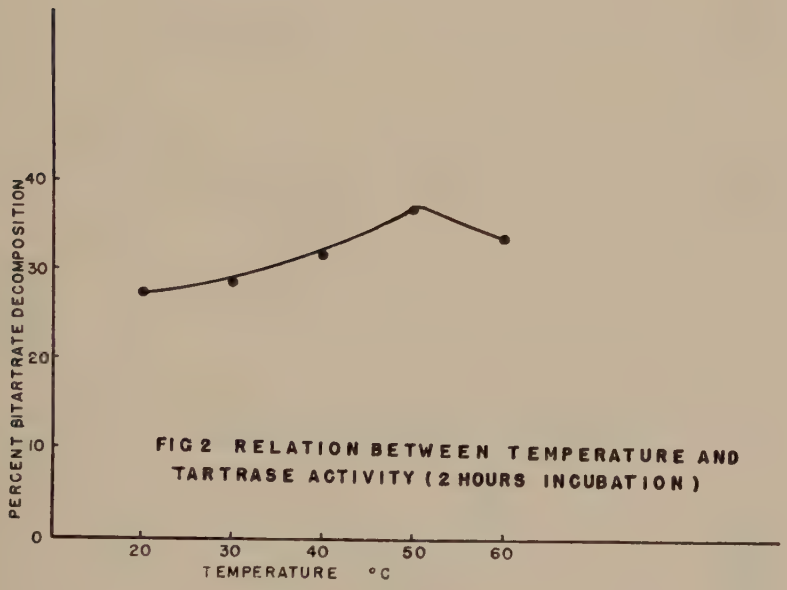

FIG 2 RELATION BETWEEN TEMPERATURE AND TARTRASE ACTIVITY (2 HOURS INCUBATION)

Mixtures of substrates with initial pH of 2.0, 3.0, 4.0, 5.0 and 6.0 and the enzyme were incubated at 30° C for four hours. As can be seen in FIG. 3, the per cent decomposition decreased significantly from a high of 45.0 per cent at pH 2.0 to a low of 23.8 at pH 6.0.

The substrates containing 0.25, 0.50, 1.0 and 2.0 per cent potassium bitartrate were incubated at 30° C for four hours. Enzymatic activity was calculated as per cent decomposition and as milligrams of tartrate per milliliter decomposition. In FIG. 4, it can be seen that per cent decomposition decreased from a high of

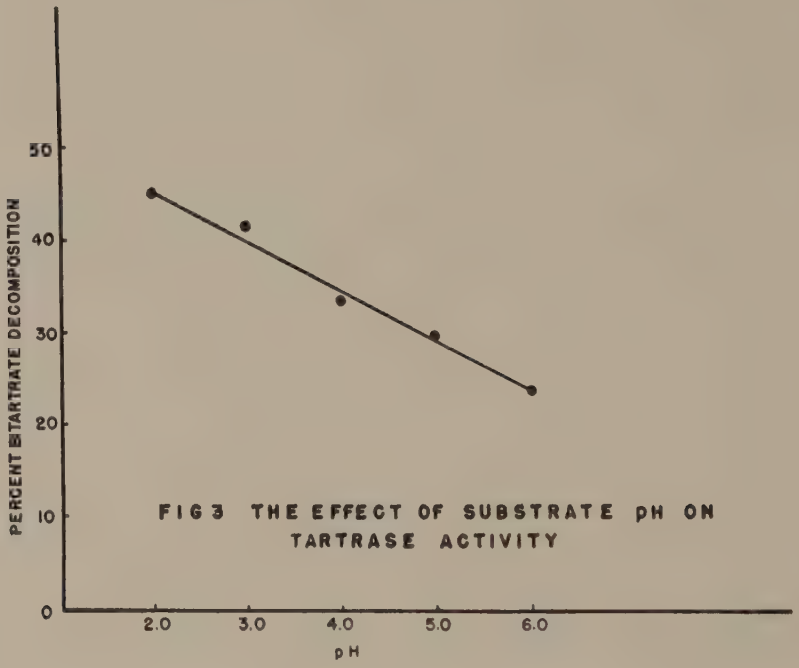

FIG 3 THE EFFECT OF SUBSTRATE pH ON TARTRASE ACTIVITY

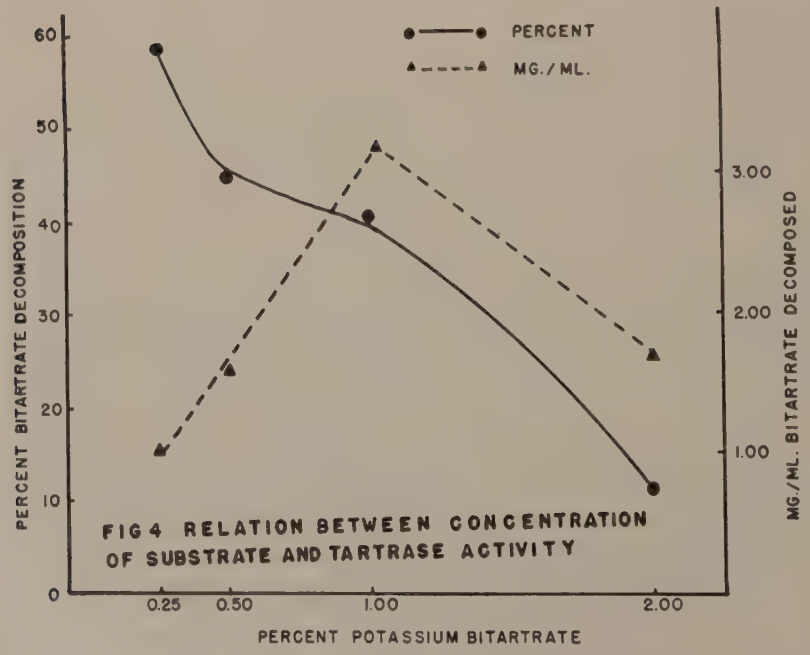

FIG 4 RELATION BETWEEN CONCENTRATION OF SUBSTRATE AND TARTRASE ACTIVITY

58.4 per cent at a substrate concentration of 0.25 per cent to a low of 11.1 per cent decomposition at a substrate concentration of 2.0 per cent. When activity was calculated as mg./ml. decomposition, it was found that activity increased from 1.05 mg./ml. at the lowest concentration to a high of 3.20 mg./ml. at a substrate concentration of 1.0 per cent and then decreased as the concentration of the substrate continued to increase.

This study was made on several different determinations as enzyme concentrations from 0.005 to 1.0 per cent were used to ascertain whether enzyme concentration influenced the rate of reaction. It was found that tartrase activity was significantly higher when used

TABLE VII

RELATION BETWEEN CONCENTRATION OF ENZYME AND TARTRASE ACTIVITY[1]

Enzyme concentration, per cent	Initial tartrate concentration, mg./ml.	Final tartrate concentration, mg./ml.	Per cent tartrate decomposition[2]
0.005	8.8	6.6	29.6
0.010	8.2	5.5	32.9
0.050	8.2	5.6	31.7
0.100	8.2	5.6	31.7
0.150	8.2	5.6	31.7
0.200	8.0	5.2	34.9
0.500	8.8	5.6	32.9
1.000	8.8	6.3	28.4

[1] As determined after incubation at 30° C for 20 hours.
[2] Average of three replications.
LSD 1.84 and 2.55 at 5% and 1% levels.

in concentrations ranging from 0.01 to 0.50 per cent than when used at higher and lower concentrations. The highest activity (34.9 per cent decomposition) occurred when the enzyme was used in a concentration of 0.20 per cent (TABLE VII).

Identification of enzymatic breakdown product of tartaric acid. After the chromatograms were developed, the substrate unknowns had separated into two definite spots. As can be seen in TABLE VIII, the Rf values of these spots checked very closely with those of tartaric and succinic acids.

Action of tartrase in Concord grape juice. When tartrase decomposed more than 30.0 per cent of the tartrates in a pure solution, there was no formation of potassium bitartrate crystals.

TABLE VIII

FILTER PAPER CHROMATOGRAM RESULTS OF IDENTIFICATION OF ENZYME BREAKDOWN PRODUCTS OF TARTARIC ACID[1]

Acid	Rf value		
	1	2	3
Succinic	0.804	0.760	0.759
Fumaric	0.886	0.852	0.875
Tartaric	0.189	0.188	0.175
Unknown No. 1	0.196	0.180	0.171
Unknown No. 2	0.800	0.768	0.761

[1] Determination made on tartaric acid substrate after 20 hours incubation at 40° C.

However, enzyme activity was erratic in decomposing the tartrates present in Concord grape juice. When tested on grape juice of the 1948 season, tartrate decomposition ranged from 24.5 to 38.2 per cent. In tests made on 1951 season juice, tartrase activity ranged from 0 to 21.2 per cent decomposition. These low activities were due to the presence of an inhibitor. Mixing small quantities of ion-exchange resins with the tartrase before addition to the juice only partially removed the inhibitor.

DISCUSSION

It was shown that a strain of *A. versicolor* could produce a tartrate decomposing enzyme sufficiently active to prevent the formation of potassium bitartrate crystals in a solution containing this salt in approximately the concentration that it is found in grape juice. That the form in which the tartrates are present influences the activity of tartrase can be seen in that tartaric acid was decomposed to a greater extent than is potassium bitartrate. As in the case of other fungal enzymes (Foster, 4; Harter & Weimer, 7), tartrase activity varies with the strain of the organism producing it. Tartrase is an adaptive enzyme (Virtanen, 18), as indicated by the fact that its activity decreases if the fungus from which it is extracted is not cultured on a medium containing potassium bitartrate as a major source of carbon.

The activation of tartrase by metallic salts is in agreement with the work of Linderstrom-Lang (18); Baumann and Heumuller

(1); and Smith and Bergman (13) on the activation of enzymes by metallic salts.

The effect of time, temperature and concentration of substrate on tartrase activity is similar to their effects on other enzymes (Gortner, 6). While there is probably an optimum pH for tartrase action, it cannot be shown using potassium bitartrate as a substrate. This is due to the fact that as the pH decreases, increasing amounts of this salt are converted to tartaric acid. As has been shown above, tartrase is more effective in decomposing tartaric acid than potassium bitartrate. While the concentration of tartrase influences enzyme activity, this influence is not proportional to the enzyme concentration as reported by Gortner (6).

Despite the fact that tartrase preparations have been obtained that prevent the formation of potassium bitartrate crystals from forming in pure solution, it cannot be used to prevent argol formation in grape products. This is due to the fact that an unidentified inhibitor is sometimes present in grape juice which either limits or stops tartrase actvity. It will be necessary to identify this inhibitor and find a way to remove it before tartrase can be used successfully in grape products.

SUMMARY

1. An enzyme extracted from *Aspergillus versicolor* cultured on grape pomace was able to prevent potassium bitartrate crystals from forming in a pure solution of this salt.

2. The enzyme activity varied with the strain of *A. versicolor* that produced it and with the form in which tartrates were used. Tartrase was more effective against tartaric acid than against potassium bitartrate.

3. Tartrase activity was increased when iron, zinc, copper, molybdenum, magnesium, or manganese salts were present in the substrate. Of these iron was the most effective in increasing enzyme activity.

4. Enzyme activity was highest at an incubation time of two hours, at a temperature of 50° C, and at a 1 per cent potassium bitartrate concentration. Tartrase activity decreased as the pH of the substrate increased from 2.0 to 6.0. The optimum enzyme concentration was 0.20 per cent.

5. It was found that in the enzyme-catalyzed reaction, tartaric acid was decomposed to succinic acid.

6. Because of the presence of an inhibitor in some grape products tartrase activity is erratic and cannot be depended upon to prevent the formation of argol crystals in grape products.

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AN UNDESCRIBED FUNGUS CAUSING A ROOT ROT OF RED CLOVER AND OTHER LEGUMINOSAE [1]

J. W. Gerdemann

(with 7 figures)

Many species of fungi are associated with red clover, *Trifolium pratense* L., root rots. One species which appears to be undescribed has been frequently isolated from diseased root specimens collected in Illinois and has been proven to be pathogenic (3). The fungus also has been isolated by the writer from dead red clover plants collected at Beltsville, Maryland, and Dr. R. G. Henderson reports having isolated it in Virginia from diseased roots of alfalfa, *Medicago sativa* L.

SYMPTOMS AND HOST RANGE

Pathogenicity tests were conducted in the greenhouse in steam sterilized, artificially infested soil. The mycelial and sclerotial growth from one agar slant was added to each 6-inch pot of soil at the time the seed was planted.

Emergence in infested pots of red clover and alfalfa was markedly reduced indicating pre-emergence damping-off. Post-emergence damping-off occurred in both species. Later the fungus penetrated the unwounded roots directly and caused a dark decay of the lateral root system and the taproot.

Preliminary experiments with this fungus indicate a wide host range within the Leguminosae. It has proven pathogenic on the following species: *Glycine max* (L.) Merr., *Lespedeza stipulacea*

[1] The writer wishes to express his appreciation to Dr. W. W. Diehl for assistance in checking literature and herbarium specimens; to Dr. Leland Shanor for helpful suggestions; and Dr. F. D. Lazenby of the Classics Department of the University of Illinois for the Latin diagnoses.

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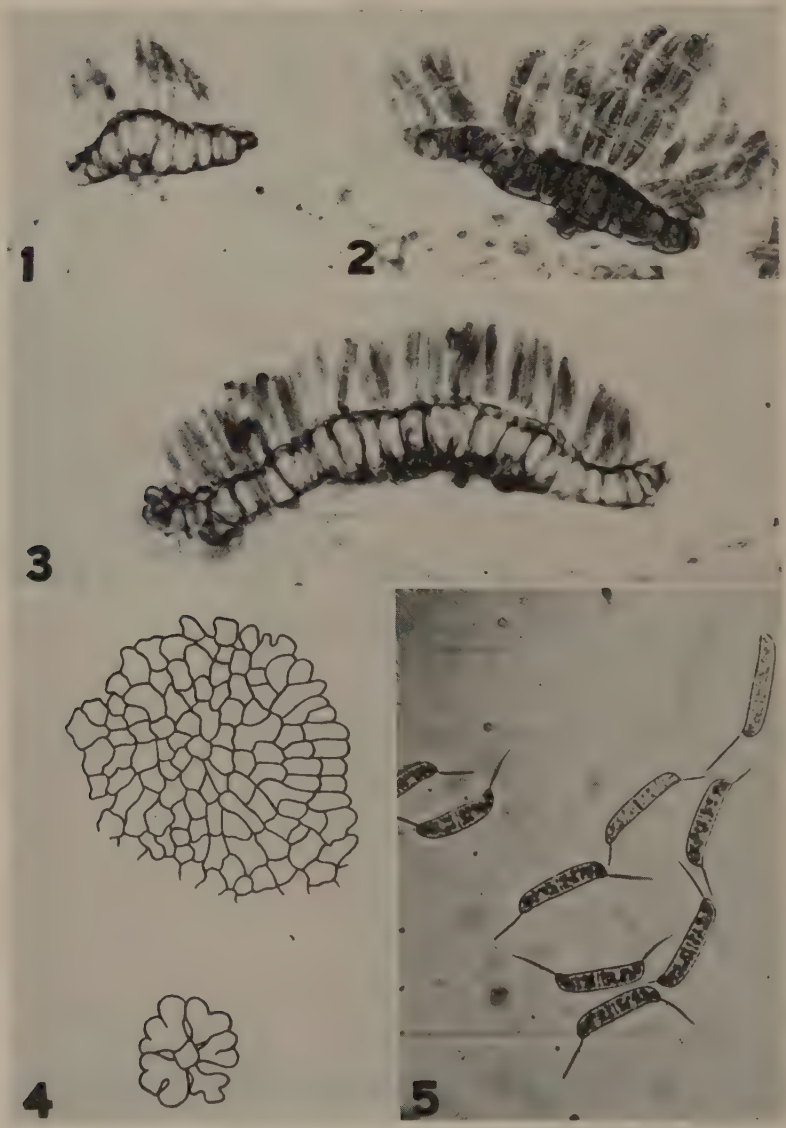
Maxim., *Lotus corniculatus* L., *Medicago sativa* L., *Melilotus alba* Desr., *Pisum sativum* L., *Trifolium hybridum* L., *T. incarnatum* L., *T. pratense* L. and *T. repens* L.

THE ORGANISM

Sclerotia are common in decayed red clover roots collected in the field and are produced in abundance on agar media. The imperfect fruiting stage has not been observed under field conditions but has been produced in the greenhouse on seedlings killed by the fungus. Fruiting has been obtained in culture on natural media sterilized with propylene oxide (**5**). Leaves and petioles of alfalfa, red clover, soybean, bluegrass or oats constitute a satisfactory medium for obtaining sporulation. The relation between the sclerotial stage and the imperfect fruiting stage was demonstrated by the use of single-spore cultures.

Sclerotia (FIG. 7) are black, spherical to fusiform in shape and may be as much as 1 mm. in length. The fruiting body (FIGS. 1, 2, 3, 4, 6) is superficial, yellow to dark brown in color, developing radially from a central cell to form a thin stroma, one cell layer thick, peltate, often fusing to form irregular plates as large as $200 \times 800\,\mu$ in diameter. Conidiophores are obsolete. Conidia are produced on the upper surface of the stromatic cells and are formed in linear rows in mucus. The mass constitutes a light yellow to brown head. The conidial walls are hyaline, but the cell contents are light yellow, often becoming brown as the spores age. Conidia (FIG. 5) are 1-septate when mature, allantoid in shape, with a filamentous seta at each end. The conidia measure 20–34.8 × 4.4–7 μ, the setae 8.7–18 μ in length.

The radial stroma often bears a superficial resemblance to the scutellum of members of the Micropeltaceae. The fruiting body might be defined either as a sporodochium or an acervulus. Employing the system of von Hoehnel (**6**) the superficial nature of the fruiting body would classify it as a sporodochium. Clements and Shear (**2**), however, regard the sporodochium as having developed from a compacting of hyphae and conidiophores, and state that the Tuberculariaceae, if properly limited, would comprise only those genera with long or branched conidiophores. They regard



FIGS. 1, 2, 3. Cross sections of acervuli of three sizes showing the one cell thick stroma upon which the spores are borne. × 455. 4. Camera lucida drawing of the spore-bearing upper surface of a young and an older acervulus illustrating the radial development from a central cell. Approx. × 550. 5. Conidia × 400.

the acervulus as a reduced fruiting body with short or obsolescent conidiophores and state that the distinction drawn by von Hoehnel with respect to the insertion of the spore body may possess some validity, but is not a practicable criterion. The writer follows the view of Clements and Shear and regards this structure as most like an acervulus.

The setulate conidia of the legume fungus are suggestive of the genus *Discosia* (4). *Discosia,* however, differs in that the conidia

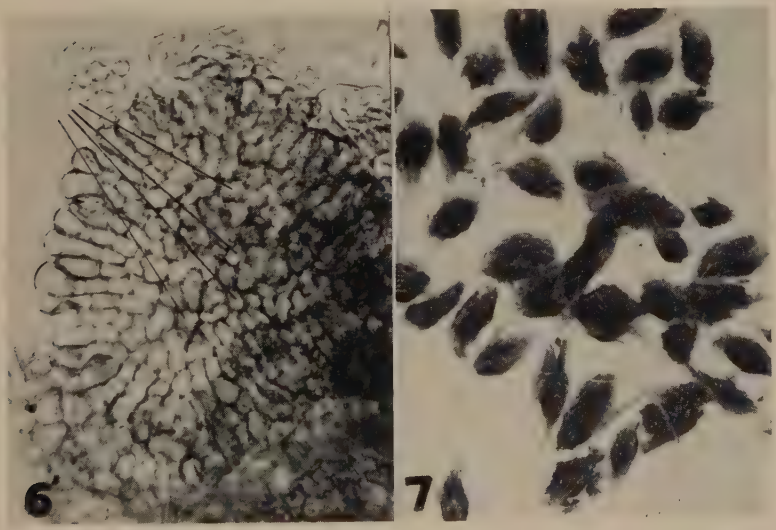


FIG. 6. Upper surface of an acervulus that resulted from the fusion of several acervuli at early stages in their development. Inked lines designate probable centers of origin. × 455. 7. Sclerotia from dead red clover roots collected in the field. × 15.

are 2- or more septate, the setae are borne to one side of the apex of the conidia, and the conidia are produced on conical conidiophores within black pycnidia. The 1-septate conidia of *Discosiella* (11) more closely resemble those of the legume fungus; however, *Discosiella* differs in that the conidia are borne on short hyaline conidiophores within completely enclosed black pycnidia that open by an irregular rupture. The conidia of *Dinemasporiella* (10) resemble those of the legume fungus but they are also borne within black pycnidia. *Pseudolachnea* (9) produces hyaline 1-septate

conidia with a seta at each end. However, they differ in that they are produced on branched conidiophores in black setulate pycnidia that are first enclosed but become cup shaped as they mature. *Dinemasporium* (4) produces hyaline 1-celled conidia that are setulate at each end. They are borne on linear conidiophores in cup-shaped black setulate pycnidia. The conidia of *Didymothozetia* (8) are 1-septate, 1-setulate at each end; but they are borne singly on ovoid conidiophores on globose hyaline sporodochia, and the setae are attached somewhat laterally. The legume fungus is distinguished from the above genera by the production of conidia directly from the cells of a superficial, one cell thick, radially developing stroma that is completely open at all stages. In addition the legume fungus has a prominent sclerotial stage.

The original description of *Pseudodiscosia* (7) refers to *Discosia*-like conidia produced directly from the cells of an acervulus. Later Buddin and Wakefield (1) reported conidiophores as present and their illustration of conidia bears little or no resemblance to conidia of *Discosia*. Grove (4) states: "It was said to resemble *Discosia* somewhat in its spores but that was a misconception." A genus having the characteristics of the legume fungus has apparently not been described and it becomes necessary to establish a new genus.

Leptodiscus gen. nov.

Sclerotia parva, nigra, sphaeralia vel fusiformia; acervuli superficiales, flavi vel fusci, scutiformes, qui e cellulo centrali radialiter orti stroma tenue efficiunt ad mensuram unius celluli crassum; conidiophora evanescentia; conidia lineari ordine in muco capitata; conidiorum parietes hyalini; conidia uno septo, una setula utrinque praedita.

Leptodiscus terrestris sp. nov.

Sclerotia nigra, sphaeralia vel fusiformia usque ad 1 mm. longa; acervuli superficiales, flavi vel fusci, scutiformes, qui e cellulo centrali radialiter orti stroma tenue efficiunt ad mensuram unius celluli crassum, et saepe inter sese nullo ordine concrescentes stroma efficiunt usque ad $200 \times 800\,\mu$ diametro; conidiophore evanescentia; conidia in summo stromate nata, lineari ordine in muco capitata; conidiorum parietes hyalini quod eis intercluditur

decolor vel flavum, interdum ad fulvum vergens; conidia cum adoleverint uno septo praedita, allantoidea, una setula utrinque praedita, 20–34.8 μ longa, 4.4–7 μ lata. Setae 8.7–18 μ longae.

The type is represented by a single-spore culture isolated from a diseased red clover root collected on the Agronomy South Farm of the Illinois Agricultural Experiment Station, Urbana, Illinois, in September, 1951. Material of the type has been placed in the Mycological Collection of the U.S.D.A. Bureau of Plant Industry, Soils and Agricultural Engineering, Beltsville, Maryland, and the Herbarium of the University of Illinois, Urbana, as Mycological Collection No. *31, 238*.

The fungus has been collected in different areas of Illinois, at Beltsville, Maryland, and Richmond, Virginia.

SUMMARY

An undescribed fungus causing damping-off and root rot of various Leguminosae is described and placed in a new genus, *Leptodiscus*. The genus is distinguished by the production of small black sclerotia, the superficial, thin radiate stroma upon which the spores are borne, and by the 1-septate conidia which have one filamentous seta at each end. The species has been designated as *Leptodiscus terrestris*.

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POLYOZELLUS MULTIPLEX AND THE FAMILY PHYLACTERIACEAE

Rokuya Imazeki

The first record of the collection of *Polyozellus multiplex* in Japan was made by Z. Tashiro at Mt. Kirisima in Kyusyu, in September, 1920. A part of the collection was sent to C. G. Lloyd by A. Yasuda. Lloyd was intuitively impressed at its being a curious foliaceous pyrenomycete and described it under the name *Phyllocarbon Yasudai* Lloyd. It is needless to say, however, that he could not find a trace of perithecia. He also mistook the basidiospores for conidia. He then noted as follows: "There is no evidence of perithecia but no doubt they will be found at a later stage as most Pyrenomycetes have an earlier, conidial stage. The plant was sent as a Basidiomycete, but it is a safe proposition that no fungi of carbonous tissue are Basidiomycetes." The plant, however, is not carbonous at all, as stated by Lloyd.

There is preserved in the herbarium of the National Science Museum in Tokyo the duplicate type specimen of *Phyllocarbon Yasudai*. The present writer has previously noticed this misreference of Lloyd's in 1938,[1] in reporting the occurrence of this rare fungus in Japan from a specimen collected near Tokyo.

The fungus was described as *Cantharellus multiplex* by Underwood in 1899 and then transferred by Murrill to his new genus *Polyozellus*. From the time of the original collection, no one recorded the occurrence of this fungus from anywhere outside of the type locality until 1937, when Mounce and Jackson reported it from Quebec in Canada. They discussed it under the original name of Underwood. In 1938 Shope noted its occurrence in Colorado, but transferred it to the genus *Craterellus*. The present writer, as noted above, reported its discovery in Japan; he

[1] R. Imazeki. A rare fungus, Polyozellus multiplex (Underwood) Murrill [in Japanese]. Nat. Sci. and Museum, IX, **10**: 3–6. 1938.

proposed that the fungus should be separated from both *Cantharellus* and *Craterellus,* and Murrill's genus should be adopted for it. Recently (1947) Smith and Morse treated the fungus in their studies in "The Genus Cantharellus in the Western United States." They considered the fungus a unique species of *Cantharellus* and provided the new section *Polyozellus* for the species, not separating it from the genus.

The fungus is also very similar to *Craterellus* in general appearance, as Shope mentioned. Murrill had, however, separated the genus *Polyozellus* from *Craterellus* on the grounds that the fruit bodies of the new genus are compound whereas those of *Craterellus* are simple. Of this treatment, Shope considered that the compound fruit bodies and the rugose hymenium are in part typical of the genus *Craterellus,* and therefore considered the genus *Polyozellus* to be superfluous.

The present writer has a different opinion. Notwithstanding the external resemblance of the hymenophore, there is no genetic relation between *Cantharellus* or *Craterellus* and *Polyozellus,* but we may find a close resemblance between *Polyozellus* and the *Thelephora* group. The most important characteristic of *Polyozellus multiplex* is represented by the spore form. The subglobose and tuberculate spores are never found in any known species of the *Cantharellus* group. They are, however, representative of the *Thelephora* group. In addition to this, the present fungus has a dark violaceous or almost black color and a very strong odor, particularly in the dried condition. Such a deep color of the fruit body and the strong odor are very common in the *Thelephora* group, but never met with in the *Cantharellus* group. Thelephoric acid is said to be a characteristic pigment of the genus *Thelephora,* and is also contained in *Calodon ferrugineus.* The writer does not know whether this substance has a strong odor or not, but he is aware of many species among *Thelephora, Calodon,* and *Sarcodon* which are dark purplish brown in color and have a unique drug-like odor, especially in dried specimens. A similar odor is strongly given off by *Polyozellus multiplex.*

From these facts, the writer has thought of this present fungus as having affinity with the *Thelephora* group. Very recently,

Miss M. Sawada [2] proved the presence of thelephoric acid in *Polyozellus multiplex*. The writer's idea is now endorsed by this fact. In conclusion, the writer came to believe that *Polyozellus multiplex* has a natural relationship with the *Thelephora* group but not with the *Cantharellus* group, although the configuration of the hymenophore might suggest the opposite.

The writer believes that the configuration of the hymenophore should not be so highly estimated as of phylogenetic significance as is held by the conservative systematic mycologists. The most primitive form of the hymenophore is smooth and even in every taxonomic group. Then, if any morphological modifications take place, it is natural that they should tend to assume the warty, tuberculate, rugulose, venulose, or reticulate appearance, and finally attain the spinulose, porose, or lamellate form. It is logical to say that the configuration of the hymenophore does not represent a phylogenetic difference between the higher taxonomic groups, but merely a lower difference within each taxonomic group. There are many examples to verify the facts among many taxonomic groups. For example:

Tremellodon, Protohydnum, Protomerulius etc. in the Tremellaceae.

Lenzites, Irpex etc. in the Polyporaceae.

Hydnochaete, Hymenochaete, Cyclomyces etc. in other brown polypores.

Paxillus, Phylloporus etc. in the Boletales.

Filoboletus, Poromycena, Dictyopanus etc. in the Agaricales.

Thelephora, Calodon, Sarcodon, Boletopsis etc. in the Phylacteriaceae.

On these grounds, the genus *Polyozellus* should not only be thought valid, but also its natural taxonomic position should be transferred to the *Thelephora* group. For the group, Donk established the subfamily Phylacteroideae in the family Aphyllophoraceae in 1933, emending the conception of Phylacteriés of Patouillard. He included in his subfamily three tribes—Thelephorinae,

[2] Sawada, M. Studies on pigments in fungi (I). On the distribution of thelephoric acid in fungi [in Japanese with English resume]. Journ. Jap. Forestry Soc. **34**: 110–113. 1952.

Hydnelleae, and Boletopseae. The writer agrees with his idea, but he proposes to give a further emendation to this group, raising it to the rank of family and adding a new tribe Polyozelleae.

Family **Phylacteriaceae** nom. nov.

Phylacteriacées Konr. & Maubl., Icon. Sel. Fung. **6**: 494. 1937.

Phylactéries Patouillard, Ess. Tax. 117. 1900—Bourdot & Galzin, Hym. Fr. 446. 1927.

Phylacteroideae Donk, Rev. Nied. Homob.-Aphyll. II, in Meded. Bot. Mus. Herb. Univ. Utrecht **9**: 24. 1933.

Hydnaceae Fries *et al.*, pr. p.

Thelephoraceae Fries *et al.*, pr. p.

Fructificatio resupinata, effuso-reflexa, pileato-sessilis vel stipitata, in forma sessili effuso-reflexa vel dimidiata, in forma stipitata unbelliformis vel ramoso-clavariformis vel polypileata (ut in *Grifola frondosa*); stipes simplex sed ramosissimus in *Thelephora* et *Polyozello*, contextus hypochnoideus, carnosus, coriaceus, generatim fusco- (brunneo- vel nigro-) coloratus, per exceptionem albus, exsiccatus saepe fortiter odoratus; hymenophorum laeve, tuberculatum vel spinulosum, raro porosum rugulosumque; sporae hyalinae, generatim coloratae, subglobosae et angulatae vel verrucosae; hyphae ad septa nodosae.

The Phylacteriaceae thus emended is a very natural group, but it is not easy to express descriptively the clear conception of this family, because the only characteristic common to all the species of the family is the globose and verrucose or tuberculate character of the spores. The stichobasidial type is regarded by Donk as an important character of the family. In *Thelephora*, the basidia are stichobasidial and arranged protohymenially. But this feature is not constant in other tribes. Spores are usually colored but sometimes hyaline. The presence of thelephoric acid is one of the noteworthy characters, which has been investigated in many species of *Thelephora* and also in *Calodon ferruginosus*. As noted above, Miss M. Sawada recently tested its presence in *Sarcodon aspratus*, *Calodon graveolens*, and *Polyozellus multiplex*. The writer is sure that this pigment will be found to occur in many other species of this family in the future. The strong drug-like odor is very

frequently met with in *Thelephora*, *Sarcodon*, *Calodon*, and *Polyozellus*, which are deep-colored species.

The family is divided into the following four tribes.

Tribe 1. THELEPHORINAE Donk, Meded. Bot. Mus. Herb. Univ. Utrecht **9**: 25. 1933.
Phylacteriacées Konrad et Maublanc, Incon. Sci. Fung. **6**: 494. 1937.

Fructificatio resupinata in generibus primitivis (*Caldesiella*, *Tomentella* et *Kneiffiella*), pileato-stipitata vel ramoso-clavariformis in *Thelephora;* contextus floccosus, membranaceus vel coriaceus; semper obscuriter coloratus; hymenophorum laeve vel tuberculosum, raro spinulosum (in *Caldesiella*).

Genera included: *Kneiffiella* Karsten; *Tomentella* (Pers.) Pat.; *Caldesiella* Sacc.; *Thelephora* Fries (*Phylacteria* Patouillard).

Since the time of the publication of the "Essai taxonomique" by Patouillard, the French mycologists have generally followed him in the arrangement of higher fungi. There is much disagreement in the interpretation of certain Friesian genera among the modern taxonomists of France and other countries. The genus *Thelephora* is one of them. The name *Thelephora* sensu Patouillard is applied to *Stereum pallidum* (*Thelephora pallida* Fr.) and its allies by some, while it is used for the group including *Thelephora terrestris* by others. Patouillard and his followers considered that the genus *Thelephora* was based on *Thelephora pallida* Fr., and he gave, therefore, the name *Phylacteria* to *Thelephora terrestris*, which has brown, verrucose spores and papillate hymenophore. On the other hand, Burt and Donk designated *Th. terrestris* as the type species of the genus *Thelephora* Fr. sensu Fr., 1821, and maintained that *Thelephora* should be used for the group with brown verrucose spores and papillate hymenophore, as had been done for a long time.

To avoid confusion in nomenclature, the writer wishes to use the name *Thelephora* in the sense of Burt, Donk, and most other mycologists, if there occurs no insuperable obstacle to doing so. As Donk noticed, Fries gave for *Thelephora* the following diagnosis and notes in his Systema Orbis Vegetabilis (1825): "Hymenium subpapillosum, inaequabile. Sporidia quaternata, fuscopurpurea" and "Cum hujus generis typo, *Thelephorae terrestri,*

nomen genericum tribuerit Ehrhartus, sensu primi determinatoris servare coactus fui." These words represent very clearly the concept of the genus *Thelephora* sensu Fries, in the meaning customarily used. Fries, moreover, apparently indicated *Thelephora terrestris* [Ehrh.] Fries as the type species of the genus.

It is not only difficult but nearly impossible to secure the real idea held by Fries directly from such an extremely complicated, heterogeneous genus as *Thelephora* as presented in the Systema Mycologicum. However, Fries's intention is clarified in the Systema Orbis Vegetabilis, as shown above. From the etymological standpoint, the name *Thelephora* means papillate hymenophore which is one of the representative characteristics of the "*terrestris*" group.

From these facts it does not seem illegal nor illogical to consider that the kernel of the genus *Thelephora* Fries of 1821 was *Th. terrestris* and its allied species, but not others.

Tribe 2. HYDNELLEAE Donk, Meded. Bot. Mus. Herb. Univ. Utrecht **46**. 1933.
Sarcodontées Konrad & Maublanc, in Fam. Hydnaceae, Icon. Sel. Fung. **6**: 492. 1937.

Fructificatio stipitata, terrestris; stipes simplex; pileus carnosus vel coriaceus; hymenophorum distincte spinulosum.

Genera included: *Calodon* Quélet (*Hydnellum* Karst., *Phellodon* Karst., *Hydnum* Auct. plur., pr. p.); *Sarcodon* Quélet (*Hydnum* Auct. plur., pr. p., *Phaeodon* Schroet.).

Tribe 3. **Polyozelleae** trib. nov.

Fructificatio terrestris merismatoidea ut in *Grifola frondosa;* contextus carnoso-mollis, in exsiccato fragilescenti rigidesceus, nigricante coloratus; hymenophorum rugulosum ut in *Cantharello.*

This tribe contains only a monotypic genus *Polyozellus* Murrill, N. Am. Flora **9**: 171. 1910.

Polyozellus multiplex (Underwood) Murrill, N. Am. Flora **9**: 171. 1910.
Cantharellus multiplex Underwood, Bull. Torr. Bot. Club **26**: 254. 1899; Smith and Morse, Mycologia **39**: 502. 1947.
Craterellus multiplex Shope, Mycologia **30**: 373. 1938.
Phyllocarbon Yasudai Lloyd, Myc. Writ. **6**: 1066, f. 2003. 1921.

Nom. jap.: Karasu-take, Karasu-maitake.

Distr.: U. S. (Maine, Oregon, and Colorado); Canada (Quebec, British Columbia); Japan; and Korea (according to Mr. Iwade).

Materials examined: Specimens of Herb. Nat. Sci. Mus. (Mt. Kirisima, leg. Z. Tashiro, Sept. 5, 1920, **type** of *Phyllocarbon Yasudai* Lloyd; Asakawa, Pref. Tokyo, leg. R. Imazeki, Oct. 25, 1936); Specimens of Herb. For. Exp. St. (Asakawa, Pref. Tokyo, leg. S. Toki, Oct. 17, 1947 and May 19, 1948).

Tribe 4. BOLETOPSEAE Donk, Meded. Bot. Mus. en Herb. Rijks Univ. Utrecht **9**: 64. 1933.

Fructificatio stipitata, carnosa; hymenophorum porosum. Genus contained: *Boletopsis* Fayod (*Boletopsis leucomelas* (Pers. ex Fr.) Fayod).

The writer wishes to express his heartiest thanks to Dr. D. P. Rogers, who carefully read this paper and gave very valuable suggestions and advice, and also gave him facilities to refer to Fries's Systema Orbis Vegetabilis which was not available to him.

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ON THE STATUS OF THE GENERIC NAMES PYRENOPHORA AND PLEOSPORA

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The genus *Pyrenophora* has been variously interpreted. It has been recognized as distinct from *Pleospora* by some investigators and united with this genus by others. However, when the two genera have been united, the genus name *Pleospora* has been used by nearly all authors. Inasmuch as *Pyrenophora* Fries (1849) has priority over *Pleospora* Rab. (1854), this is incorrect usage and would involve wholesale name changing. It is of import, therefore, to determine the status of these two names.

The genus *Pyrenophora* was described by Fries (1849), as follows:

"Nucleus serotinus, grumoso-gelatinous, in stromate ceraceo indurato (Sclerotiodea), perithecii vices gerent, immersus, ostiolo tarde prominule.

"Asci firmi, sporis opacis multiseptatis Berk. Suppl. Vol. VI. tll, f. 8."

Singulare et affinitatis incertae genus, cui plura exotica habemus affinia. Cfr. Dothiorum.

Three species were given as follows:

1. *Pyrenophora paradoxa* Fr. 1 (nucl. pallideus) S. M. p. 549.
2. *P. inclusa* (Kunze sub. Sclerot.) 1.
3. *P. phaeocomes* Reb. 1, 2, B. n. 185 Grev. Scot. t. 69.

Through the kindness of J. A. Nannfeldt it has been ascertained that there are only two collections of these species, one of *P. inclusa* and one of *P. phaecomes* in the Fries Herbarium. Whether or not these are holotype material is doubtful, but they are, apparently, the only lectotype material available, or in existence.

P. paradoxa was previously described (Fries 1823) as *Dothidea paradoxa*, as follows: "subcaespitosa, nuda, elongate, difformis, rugosa, atra, intus alba." It is given as "rarissima" and on de-

nuded *Salix* wood. There appears to be no specimen in existence, and the species has been dropped from the literature.

P. inclusa was based on *Sclerotium inclusa* Schm. & Kze. (Deut. Schwamme *137*) and described by Fries (**1823**) as on poplar leaves. A collection in the Fries Herbarium consists of a single poplar leaf with a number of sterile sclerotia similar in appearance to *Sclerotium bifrons*. These sclerotia are circular, disc-shaped, black, with wrinkled upper exposed surface and are 800–1000 × 400–500 μ. There is an outer blackened crust and an inner hyaline parenchymatic cortex with some of the cells containing a yellow resin-like material, but beyond this there is no differentiation and there are no setae nor any tomentum.

Pyrenophora phaeocomes (Reb.) Fr. is the species which is usually cited in the literature as the type species of *Pyrenophora,* because the two previous species were poorly known. *Sphaeria phaeocomes* was described by Rebenitsch (**1804**) as follows: "simplex, sparsa, epiphylla, globosa hemisphaeriis, utrinque folii pagina pilis erecto-divergentibus obsitis. R. Tab. 1, fig. 4a, 4b. In *Holci mollis* foliis languescentibus. Automno.

"Sphaeria atra duriuscula globosa, intus albida, utrinque folii pagina pilis oblita. E. longinquo *Dematium* effe videtur at accuratius adspecta et transversim perscissa ab eo facile diagnoscitur."

His figures are merely rough sketches of the type of setose sclerotia usually associated with this species. There is a collection of this species in the Fries Herbarium consisting of five or six stems and leaves of some grass which contain a number of sclerotial ascocarps typical of such species as *Pyrenophora trichostoma* (Fr.) Fck. and *P. phaeocomes* (Reb.) Fr. They are 350–500 × 250–400 μ in diameter, somewhat depressed-globose, buried in the leaf tissue, finally erumpent-superficial, and bear a crown of dark brown, tapered, sparingly septate, stiff, setose hyphae, up to 70 μ in length and 7–12.5 μ in diameter. There is an outer blackened wall or rind and an inner hyaline parenchymatic cortex, but all of the 12–15 fruit bodies examined were completely sterile.

From the preceding it can be seen that all of the Friesian type material consisted of sterile sclerotia (and probably also all pre-

Friesian material cited by Fries), and according to Art. 57, as revised at the Stockholm Congress, the name *Pyrenophora* would refer to this sterile state and not be valid for an ascus or perfect genus.

Fries's description also refers only to sterile sclerotia, except that portion which is placed in quotation marks, and apparently refers to the Berkeley (**1841**) reference, which figures spores and asci. As a matter of fact, Berkeley's figures do not represent the asci and spores of Fries's sclerotial fungus, as will presently be shown, and if this is accepted as a part of the description of the type of *Pyrenophora* then this type, *P. phaeocomes,* consists of a mixture of "two or more entirely discordant elements" as mentioned in Art. 64 of the International Rules, and the only choice of a "satisfactory type" would be the sclerotial stage of Fries's specimens of *P. phaeocomes.*

In the reference mentioned by Fries, Berkeley (**1841**), in a discussion of *Diplodia viticola* Desm., referring to the method of cavity formation in the stromatic pycnidium, says: "This is the case with certain species of *Sphaeria,* which will hereafter probably constitute a new genus. Amongst them is the curious *S. phaeocomes,* which I have found in fructification and of which I give a figure." The figure (Pl. 11, fig. 8) presents a few asci and spores, which have three transverse and one or more vertical septa, but appear much smaller than those typical of the perfect stage of Fries's sclerotia. Berkeley (**1860**) listed the genus *Pyrenophora* and gave the spores as "multiseptate" and listed one species, *P. phaeocomes* Fr.

The earliest collection of *Sphaeria phaeocomes* Berkeley and Broome that the writer has been able to locate is found on two sheets from the Broome Herbarium, labelled *Sphaeria phaeocomoides,* in the British Museum of Natural History. The data on the sheets give the fungus as being on *Alisma plantago,* Rudloe, 6/1, – 43 and on one sheet there is a notation "Annals N. H. vol. 6 (*Pyrenophora* Fr.) (foliicola)" and figures of spores similar to those mentioned above. Although this collection was apparently made after the note was published in the Notices of British Fungi, it seems to be authenticated by the authors. This collection is of

Pleospora vulgaris Niessl. It has perithecia which are 150–200 μ in diameter and with no setae. The asci are 65–85 × 8.5–13.5 μ and the spores are 3–5-septate, oblong-ellipsoid, constricted at the septa, with vertical septa in the central cells only, and measure 15–18 × 5.5–6.5 μ. Fries's reference to the spores as "opacis" would also suggest this species rather than the ascospores of *P. phaeocomes,* which are pale yellow for some time before maturity.

The reference to Greville (**1823–29**), by Fries and Berkeley, is the description of *Cryptosphaeria capillata.* He figures setose fruiting bodies similar to those of Fries's *P. phaeocomes,* but again states: "I have not been able to discover the sporules" and refers to Nees von Essenbeck's (**1817**) *Sphaeria capillata,* who figures "ascus granules" with three septa.

Cesati and de Notaris (**1861**) listed *Sphaeria phaeocomes* Berk. & Br. under their genus *Pleospora.* Niessl (**1876**) described *Pleospora phaeocomes* Ces. & de Not. and based it upon *Sphaeria phaeocomes* Berk. & Br. (Brit. Fung. Nr. *207*). Niessl gave the species as upon *Vitis,* probably because it was discussed under *Diplodia viticola* by Berkeley, and also on Umbelliferae. He said the spores were dark-colored (as given by Fries), 5-septate, and of the form of *Pleospora media,* which agrees with the material in the Broome Herbarium. Saccardo (**1883**) transferred this species to *Pyrenophora,* recognized it as distinct from *P. phaeocomes* (Reb.) Fr., named it *P. phaeocomoides* Sacc. and properly recognized *P. phaeocomes* as based upon *Sphaeria phaeocomes* Reb., but incorrectly referred the *Pyrenophora* binomial to himself and the *Pleospora* one to Fries instead of vice versa.

Berlese (**1900**) figured the spores of these two species, showing the same differences. *Pyrenophora phaeocomoides* (from Niessl's material) shows (**1900,** Pl. 53, fig. 2) the spores of *Pleospora vulgaris* but setose perithecia. *Pyrenophora phaeocomes* (Reb.) Fr. (**1900,** Pl. 54) shows very large, 6–7-septate ascospores and the large setose perithecia as found in sterile collections of this species.

From the preceding, it is obvious that Berkeley and Broome's *Sphaeria phaeocomes* is not the same as that of Rebenitsch, but is the same as *Pleospora vulgaris* Niessl, and that it is not the ascus

stage of the large setose sclerotia of Rebenitsch's figures and Fries's collection. It also appears that the genus name *Pyrenophora* must either be considered as a form genus name (near *Sclerotium*) because of the sterile nature of the type material, or else be discarded, if Fries's reference to Berkeley's description of spores is accepted, for the description then refers to two discordant elements.

THE GENUS PLEOSPORA

There are also certain obstacles in the way of acceptance and typification of the genus name *Pleospora*, which should be cleared up. The genus name *Pleospora* was first used by Rabenhorst, upon a series of exsiccati (Herb. Myc. II, *547a-c*) issued as varieties of a species *Pleospora herbarum* Rab. On the first exsiccatus (*547a*) are the following notes:

Pleospora Rabenh. Sphaeriacerum nov. genus. Sphaeriae sp. (herbarum) Autt.
Pleospora herbarum Rab. var. *Allii* Rab. Mspt. (*S. Allii* Rab. Herb. Myc. I, 838—*S. herbarum* var. *Allii* Desm.) *Allium*, Doenitz, leg. Fischer.

The genus *Pleospora* has generally been referred to Rabenhorst on the basis of this exsiccatus. As a matter of fact, this does not constitute either a species or generic description, and although the name *Sphaeria herbarum* (Autt.) is mentioned, it is obvious that Rabenhorst was not sure of this synonymy.

The type collection of *Sphaeria herbarum* Fr., quoted by Fries (1823, 2:511) as Scler. Suec. No. *38*, has proven to contain only a small *Phoma*, both in the copy in the Uppsala Herbarium of Fries (kindly examined by J. A. Nannfeldt) and in that in the Farlow Herbarium (examined by the writer). The Persoonian type of *Sphaeria herbarum* var. *tecta*, quoted by Fries (1823, 2:511), was kindly loaned by H. J. Lam of the Leiden Rijksherbarium, and proved to be a species of *Ophiobolus*. It is obvious, therefore, that the use of the epithet *"herbarum"* for Rabenhorst's exsiccatus was a misdetermination, and that *Pleospora herbarum* Rab. refers either to a *Phoma* (if one uses the Friesian type) or to an *Ophiobolus* (if one chooses to go back to the Persoonian type). In a later exsiccatus, of the same year (Herb.

Myc. II, *731*) of *Sphacria Typharum,* Rabenhorst refers to *Sphacria scirpicola* in the following words: "sporis multicellularibus (transv. et longitudinaliter septatis) gaudet, qua de causa PLEOSPORA VERA et minime Sphaeria." This again is scarcely to be called a generic description.

The first properly published description of the name *Pleospora* was by Cesati and de Notaris (**1861**). Their description as "Sporidia ampla; plurilocularia, loculis transvers seriatis" would also exclude *Pleospora herbarum* (Fr.) Rab. as typified by Scler, Suec. No. *38*. These authors list three generic synonyms, fourteen species of *Pleospora* and five species of *Sphaeria* under this generic name. The sixth species of Cesati and de Notaris is *Pleospora Allii* which is based upon Rabenhorst's *P. herbarum* var *Allii,* which in turn is based upon *Sphaeria Allii* Rab. (as issued in Herb. Myc. I, *838*).

Inasmuch as the name *Pleospora herbarum* is attached to a Friesian type of the genus *Phoma* (or a Persoonian type of the genus *Ophiobolus*), as Rabenhorst's original use of the generic name *Pleospora* did not include a generic or specific description, and inasmuch as Cesati and de Notaris' sixth species, under the first proper description of the generic name, refers to Rabenhorst's original exsiccati (Herb. Myc. I, *838* and II, *574a*), which he obviously meant to be the basis of the genus *Pleospora,* it is proposed that the genotype of this genus (*Pleospora* Rabenh. ex Ces. & de Not.) be conserved as *Pleospora Allii* (Rab.) Ces. & de Not., with Herb. Myc. I, No. *838* as the lectotype collection of the species.

Such a disposition, as outlined, will involve only one name change, the substitution of *Pleospora Allii* (Rab.) Ces. & de Not. for *P. herbarum* (Rab.) Fr., and will establish the genus *Pleospora* on a broad enough basis to include species formerly placed in the (form ?) genus *Pyrenophora,* if so desired.

The fact remains that there are a group of species related to *Pleospora trichostoma* which have certain characters in common which, together, distinguish them from most other species of *Pleospora.* These characters are the sclerotial character of the perithecium with a thick stromatic wall, the presence of a crown

of seta-like hyphae about the ostiole, the large pale yellow-brown ascospores and the presence of a *Helminthosporium* conidial stage. The difficulty lies in the fact that no one of these characters is a constant difference. *Pleospora herbarum* and other species may have large sclerotial perithecia; the crown of setae is common in other species of *Pleospora;* some of the *P. trichostoma* group, such as *P. macrospora* on *Hierochloe,* may lack such setae; and there are, again, strains of *P. trichostoma* which do not produce *Helminthosporium*-like conidia.

The name *Scleroplea* might be available for this group, if considered a separate genus. Saccardo (**1883**, p. 277) used this name for a subgenus of *Pleospora,* with sclerotioid perithecia. He obviously considered the setose ostiole as the distinguishing character of *Pyrenophora.* Oudemanns (**1901**) raised it to generic rank and von Höhnel (**1907**, p. 635) considered it as differing from *Pyrenophora* in the lack of the ostiolar setae. The first species mentioned by Sacardo under his subgenus *Scleroplea* is *Pleospora nuda* (Cke.) Sacc. The writer has examined three portions of Cooke's type collection of *P. nuda.* They all show black sclerotial areas 300–1000 μ long, on leaves, which are entirely sterile. In the specimen at the Kew Herbarium, masses of black toruloid hyphae were seen on these black spots; the packet in the Riksmuseet showed only an accompanying *Ascochyta.* A copy in the Farlow Herbarium yielded a few perithecia with ellipsoid, muriform spores, 22–27 × 10–11 μ and 5–7-septate. This was *P. media* or *P. herbarum.* Cooke (**1879**) in his original remarks says, "there are no true perithecia, the cells surrounding the perithecial cavity are brown, globose, and readily separable." His description apparently includes a mixture of fungi. Oudemans placed his *S. cliviae* as his first species. The writer has not seen this fungus but the description and figures of Oudemans (**1901**) again suggest *P. herbarum.* It seems that if this "*Pyrenophora*" group is to be recognized, a new generic name must be created, but inasmuch as much more needs to be known concerning the details of perithecial development before the true relationships and proper disposition of species can be determined in this group, such a creation at this time does not seem practicable.

The three following species are all closely related and of this *"Pyrenophora"* type, and they are all found upon grass hosts.

PLEOSPORA TRICHOSTOMA (Fr.) Ces. & de Not. Schema sfer. ital. 217. 1861.

This species has been discussed previously (Wehmeyer **1949**). It occurs on a large variety of grass genera and has 3-septate ascospores which show a wide range in size (35–60 × 13–23 μ) and has been described under a number of species names. Both Fuckel (**1869**) and the Tulasnes (**1863**) report the spores as 3–4-septate but the writer has not seen such spores with more than three septa and they are probably unusual, except, perhaps, upon germination.

PLEOSPORA PHAEOCOMES (Reb.) Wint. in Rab. Krypt. Fl. Deutsch. etc. **1**(2): 521. 1887.

Sphaeria phaeocomes Reb. non Berkeley, Prodr. Fl. Neomarch. 338. 1804.

Pyrenophora phaeocomes (Reb.) Fr. Summa Veg. Scand. 398. 1849.

This species differs in the larger spores (40–100 × 20–41 μ) which have 4–6 (–7) septa. The perithecial sclerotia of this species remain sterile for a long period of time and are so found in many collections and exsiccati. Fuckel (**1869**) seems to have been the first to describe the spores of this species, which he states are 3-septate at first, then multiseptate and muriform. His figure (Pl. 6, fig. 31) shows a 3-septate spore with appendages and without vertical septa, presumably a young spore (?). Berlese (**1901**, Pl. 54) gives good figures of the spores of this species. Rebenitsch, Fuckel, Winter and Berlese all report this species on *Holcus*. A collection of this species, with 6-septate spores, on *Anthoxanthum* was sent to the writer by Dr. L. Holm from Sweden, and Müller reports it upon *Calamagrostis* from Switzerland.

PLEOSPORA MACROSPORA Schroet. Hedw. **2**: 153. 1882 (Jahresber. d. Schles. Ges. d. Vaterl. Cultur. 1880).

Clathrospora macrospora (Schroet.) Nannf. Mitt. Bot. Inst. Techn. Hochschühle Wien **8**: 30. 1931.

This species appears to be common in northern Europe on *Hierochloe,* but has also been found by Mr. C. B. Kenaga, near

Ann Arbor, Michigan, on *Poa sp.* It is very similar to *Pleospora trichostoma* in its 3-septate spores, which, however, are toward the lower end of the size range (30–45(–50) × 10–18 μ). The perithecia are smaller than in *P. trichostoma* and are almost smooth or with a few short hair-like setae.

Pleospora scirpicola (DC.) Karst. and *P. typhaecola* (Cke.) Sacc. are also similar to the above three species, and the spores of both show an irregular flattening in one plane, which is also true to a lesser degree in the spores of *P. macrospora.*

In the writer's opinion, therefore, the following conclusions should be reached:

1. That *Pleospora Allii* (Rab.) Ces. & de Not., with Rabenhorst's Herb. Myc. I, *838,* of *Sphaeria Allii,* as the type specimen, be conserved as the lectotype of the genus *Pleospora.*

2. That the genus name *Pyrenophora* be removed from the Ascomycetes to the Fungi Imperfecti as a form genus name.

3. That until the proper taxonomic limits of the group of species about *Pleospora phaeocomes* can be determined, they be retained as a sub-group of the genus *Pleospora.*

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PUCCINIOSTELE, A GENUS OF THE RUST FUNGI [1]

GEORGE B. CUMMINS AND M. J. THIRUMALACHAR

(WITH 1 FIGURE)

This paper presents information concerning the morphology of the genus *Pucciniostele* Tranz. & Kom. (*Klastospora* Diet.). The four species thus far described are *P. clarkiana* (Barcl.) Diet., *P. mandschurica* Diet., and *P. sydowii* Liou & Wang on species of *Astilbe,* and *P. hashiokai* (Hirat. f.) Cumm. on *Ampelopsis.* An additional species, *P. philippinensis,* on *Astilbe* is described below. All are Asiatic in distribution.

CHARACTERISTICS OF THE GENUS

SPERMOGONIA AND AECIA. The spermogonia are subcuticular, applanate or lenticular in shape, and with a flat or nearly flat sporogenous layer. In size they vary from 40 to 60 μ in height and 100 to 200 μ in diameter with no marked variation between species. Some hypertrophy results from infections on *Astilbe.* The aecia are caeomoid, but in *P. philippinensis* there is present what may be a rudimentary peridium. Spore-like cells adhere to the epidermis in a peridium-like manner, are usually brownish, and have irregular markings of a labyrinthiform nature. The aeciospores are verrucose with small rounded warts and are provided initially with intercalary cells which are displaced as the spores mature. For the species on *Astilbe* the aecia at first surround and later displace the spermogonia since they occupy areas

[1] Cooperative investigations between the Purdue University Agricultural Experiment Station and the Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Journal Paper No. 660, of the Purdue University Agricultural Experiment Station. Contribution from the Department of Botany and Plant Pathology.

several millimeters in length. Aecia are not known for *P. sydowii* and *P. hashiokai.*

UREDIA. Previous to this study uredia were not recognized, *P. clarkiana* and *P. mandschurica* being considered, and probably rightly so, as *opsis*-forms. However, *P. philippinensis* develops uredia as does also *P. hashiokai,* but the two are unlike. In *P. philippinensis* the uredia are provided with a uniseriate peridium composed of more or less cubical cells which are firmly united. The peridium adheres closely to the epidermis of the host but can be separated off in sizeable sheets. Like the aeciospores, the urediospores are catenulate and verrucose. In *P. hashiokai,* however, the uredia have peripheral paraphyses as in *Phakopsora* and the echinulate spores are borne singly. The inclusion in one genus of species having such diverse uredia is open to question.

TELIA. *Pucciniostele* is unique in that two kinds of telia, primary and secondary, have been reported and do occur in the species on *Astilbe.* This has been confirmed and illustrated by Koursanov, Zeshinskaja, and Klushnikova (**4**) who studied *P. mandschurica* cytologically. The primary telia develop in the base of the aecia and completely displace the aeciospores. The primary teliospores are usually in the form of tetrads (FIG. 1, A) and arise from the same basal cells as do the aeciospores. Consequently, a single linear series may have chains of aeciospores apically and chains of teliospore tetrads basally. In later stages the sorus may contain only teliospores chains, which are sufficiently obvious to be observed with a hand lens. Lateral adherence between chains is not great but adherence between tetrads is strong. While the tetrad is commonest, teliospores may be unicellular or bicellular. Koursanov *et al.* (**4**) report that the teliospores are initially binucleate but become uninucleate by nuclear fusion. Germination of the primary teliospores has not been observed and their role in the life cycle is unknown.

Secondary telia develop later in the season and not in close association with the aecia. In *P. philippinensis,* as also in *P. hashiokai,* they are associated with the uredia. As was originally emphasized by Barclay (**1**), the telia have a superficial resemblance to those of *Coleosporium* in that they are golden and waxy in

appearance when immature but becomes nearly brown when mature. The telia consist of subepidermal crusts (FIG. 1, B) of unicellular teliospores produced catenulately. Lateral adherence of the chains is not great, and as the sorus reaches maturity they separate in varying degrees and fall from the sorus, leaving only the bounding ruptured epidermis of the host. Germination of the secondary teliospores has not been observed. While obscure, there is some evidence that there may be two or three germ pores in the species on *Astilbe* and this is certainly true of *P. hashiokai*.

THE SPECIES OF PUCCINIOSTELE

PUCCINIOSTELE CLARKIANA (Barcl.) Diet. (Engler's Bot. Jahrb. 27: 564. 1899) occurs on *Astilbe congesta, A. japonica, A. kiusiana, A. longicarpa, A. macrophylla, A. rivularis,* and *A. thunbergii* in China, India, and Japan.

The species develops spermogonia, caeomoid aecia with the aeciospores measuring 16–24(–26) × (21–)24–32(–35) μ and having an apical wall 4–10 μ in thickness, primary telia in the aecia, and secondary telia. Uredia are not known.

PUCCINIOSTELE MANDSCHURICA Diet. (Ann. Mycol. 2: 21. 1904) occurs on *Astilbe chinensis* and *A. microphylla* in China, Japan, Korea, and Manchuria.

The species forms spermogonia, caeomoid aecia with the aeciospores measuring (14–)17–24 × (18–)22–28(–30) μ and having the apical wall uniform or thickened to 4 μ, primary telia in the aecia, and secondary telia. Uredia are unknown.

PUCCINIOSTELE SYDOWII Liou & Wang (Chinese Jour. Bot. 1: 81. 1936) occurs on *Astilbe* sp. in India. Only telia are reported. It is doubtful if this species differs from *P. clarkiana*.

Pucciniostele philippinensis sp. nov.

Spermogoniis amphigenis, subcuticularibus, lenticularibus, 45–60 μ altis, 120–200(–275) μ latis, sine paraphysibus. Aeciis caeomoideis, hypophyllis vel caulicolis, magnis, frequenter confluentibus, rotundatis vel elongatis et usque ad 2 cm longis, aurantiacis; aeciosporis variabilibus sed plerumque oblongis vel oblongo-ellipsoideis, (17–)20–26(–30) × (29–)32–40(–43) μ, membranis 2–3 μ cr. ad apicem 8–15(–20) μ cr., moderate verrucosis, hyalinis. Urediis sparsis, hypophyllis, aureis, peridio ex cellulis cuboideis, 12–15 μ

FIG. 1. Types of teliospores produced by the species of *Pucciniostele* on *Astilbe*. A. Primary teliospores of *P. clarkiana*. This spore form develops in aecial sori. The characteristic tetrads of spores do not adhere laterally but are attached vertically, resulting in chains of tetrads. B. Secondary telia of *P. mandschurica* in section. These telia occur later in the season than the primary telia and are not associated with the aecial infections. The unicellular spores are strongly adherent vertically but the chains tend to separate laterally at maturity. Both kinds of telia are known in *P. clarkiana, P. mandschurica,* and *P. philippinensis*. Germination has not been observed in either type. × 800.

diam., flavidis, membranis 1.5–2 μ cr. instructis; urediosporis catenulatis, oblongis vel oblongo-ellipsoideis, 17–24 × 26–31 μ, membranis hyalinis, 2 μ cr. vel ad apicem 4 μ cr., moderate verrucosis. Teliis primariis ex aeciis ortis, teliosporis primariis irregularibus plerumque ex 4-cellulis compositis,

19–22 × 26–32 μ, cellulis singulis plus minusve cuboideis, pallide aureis; teliis secondariis sparsis, hypophyllis, subepidermalibus, aureis, rotundatis, 0.1–0.6 mm diam., teliosporis 4–8 in catenas cylindraceas connexis, sporis individuis cuboideis, 13–14 × 10–15 μ, membranis pallide flavidis, 1–1.5 μ cr.

On leaves, petioles, stems, and inflorescence of *Astilbe philippinensis* Henry in the Philippine Islands. The type of the species is Clemens No. *51787* collected on Mt. Santo Tomas, Benguet Prov., Luzon, Mar. 26, 1935.

P. philippinensis differs from *P. clarkiana* and *P. mandschurica* in several respects. Uredia apparently occur only in *P. philippinensis.* It has the largest aeciospores and the greatest apical thickening of any of the species of the genus. In these respects the species form a series from large spores to small spores as follows: *P. philippinensis, P. clarkiana, P. mandschurica.* Since aeciospores have not been reported in *P. sydowii* it cannot be compared. Peridium-like cells adherent to the epidermis are prominent in *P. philippinensis,* occur in some collections of *P. clarkiana,* but have not been observed in *P. mandschurica.* Spermogonia are present in all three and are much alike. No dependable differences could be found in the primary or secondary telia. The secondary telia of *P. philippinensis* were found only in immature condition so that the length that the spore chains may attain is uncertain.

PUCCINIOSTELE HASHIOKAI (Hirat. f.) Cumm. (Mycologia **42**: 790. 1950) occurs on *Ampelopsis cantoniensis* in China and Formosa.

Spermogonia and aecia are unknown in this species. It forms uredia with peripheral paraphyses and echinulate spores and secondary telia. The discovery of the aecia will be necessary before the relationships of this species can be decided. While the telia and teliospores are generally similar to those of the species on *Astilbe* the phakopsoroid uredia are much different.

DISCUSSION

The relationships of *Pucciniostele* are obscure. Dietel (**2**) and the Sydows (**6**) place the genus in the Pucciniaceae with several other genera of dubious affinities. On the other hand Kursanov

et al. (4) and Hiratsuka (3) consider that the genus belongs in the Melampsoraceae. The latter treatment seems the more logical for several reasons: 1) peridiate uredia are common in the Melampsoraceae but very rare in the Pucciniaceae; 2) catenulate urediospores occur in the Melampsoraceae but not in the Pucciniaceae; and 3) catenulately produced teliospores adherent in chains occur in the Pucciniaceae only in the Pucciniosireae, a tribe of anomalous and mostly microcyclic genera. The lack of strong lateral adherence of the chains of teliospores is the only character present in *Pucciniostela* that would indicate relationship with the *Pucciniaceae*.

In the Melampsoraceae catenulate urediospores occur in *Mesopsora* (spermogonia and aecia unknown), in *Chrysomyxa* (aecia on *Picea*), and in *Coleosporium* (aecia on *Pinus*). The teliospores also occur in chains in *Chrysomyxa* and there is a very close similarity between the aeciospores and the urediospores of any one species, as pointed out by Saville (5), and this is also true of *Pucciniostele*. On the other hand, the aecia of *Chrysomyxa* are peridermioid and the teliospores have non-pigmented walls and germinate without a dormant period. The teliospores of *Pucciniostele* are only slightly pigmented but apparently are not germinable at maturity. Moreover, the spermogonia of *Chrysomyxa* are subepidermal. *Coleosporium* has subepidermal spermogonia and peridermioid aecia and, in addition, the teliospores produce internal basidia and are not typically catenulate. If *P. hashiokai* actually belongs in *Pucciniostele,* relationship to the *Phakopsora-Angiopsora* complex is possible. As mentioned above, this species has phakopsoroid uredia. Most species of *Angiopsora* also have such uredia and produce their teliospores in chains. Aecia have been reported only for *Phakopsora hansfordii* Cumm. (*Angiopsora hansfordii* Thirum. & Kern 1949, not *A. hansfordii* Cumm. 1945). In this species the spermogonia are similar in position and morphology to those of *Pucciniostele* but the aecia have a peridium. Moreover, in *Angiopsora* and *Phakopsora* the lateral adherence of the teliospores is stronger. *Phragmidiella* has similar telia, subcuticular but paraphysate spermogonia, uredinoid subcuticular aecia, and pedicellate urediospores.

While all of these genera have some points of similarity with *Pucciniostele*, all also have sufficient differences as to make it impossible accurately to determine the actual relationships. On the other hand, the evidence does suggest strongly that *Pucciniostele* belongs in the Melampsoraceae near the genera discussed.

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TWO COMMON LUMBER-STAINING FUNGI IN THE WESTERN UNITED STATES

Ross W. Davidson [1, 2]

(with 3 figures)

In the summer of 1944 the writer obtained pure cultures of some staining fungi on coniferous lumber in Oregon and Washington. Time did not permit a thorough study of all the staining fungi encountered but it was found that there were two very common and conspicuous species. One of these, a common cause of dark stain on Douglas-fir sapwood, is very similar to the European fungus *Endoconidiophora coerulescens* Münch. The other species, on many coniferous woods, seems to be the same as the European species *Ophiostoma piceae* (Münch) Sydow. The present paper gives available information on these two fungi, neither of which is known to be present in the eastern or southeastern United States.

Ophiostoma piceae

There is commonly present on Douglas-fir sapwood and on lumber of other western species, such as western hemlock, noble fir, and Sitka spruce, a fungus with a *Graphium* stage. It is usually found on wood having no pronounced stain, or it may be present on wood stained by other fungi.

When transferred in pure culture the *Graphium* or coremium stage forms very quickly (Fig. 1, E), along with conidia borne directly on the hyphae (Fig. 1, C). The cultures on malt agar medium may become dark gray or dark brown but often remain

[1] Senior Pathologist, Division of Forest Pathology, U. S. Dept. Agr., in cooperation with Rocky Mountain Forest Experiment Station and Colorado Agricultural Experiment Station. Colo. Agr. Expt. Sta. Scientific Paper No. 396.

[2] The writer was assisted by Ernest Wright, Division of Forest Pathology, U.S.D.A., in collecting much of the material from which cultures were obtained.

light gray to light brown. After several weeks long-beaked perithecia may begin to develop slowly in the cultures (FIG. 1, A). Usually perithecia are not abundant and are mixed in with the densely formed *Graphium* fruiting bodies. Some cultures do not form perithecia.

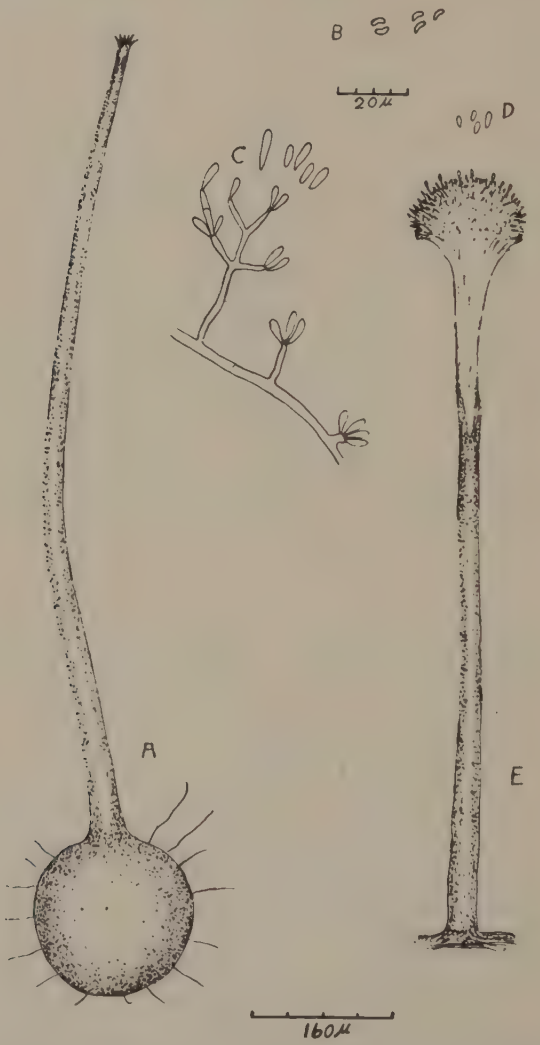

FIG. 1. *Ophiostoma piceae*. A. Perithecium; B. ascospores; C. conidia and conidiophores; D. conidia from *Graphium* stage sporophore; E. *Graphium* or coremium stage.

Morphologically this western fungus compares closely with that described as *Ophiostoma piceae*. Lagerberg *et al.* (4) state that this species develops very slowly and does not cause pronounced stain in coniferous wood. Growth rate in culture is about the same as that recorded for *O. piceae*.

The fungus is difficult to maintain in normal condition in culture on malt agar, a fact also pointed out by Lagerberg *et al.* (4). It grows well and forms abundant coremia when first isolated; but it changes when held in culture for a long period, first, by forming white to light brown sectors with few or no coremia or perithecia, and later, the entire culture may revert to a non-fruiting condition. This is also true for many of the wood-staining fungi, so that it is difficult to study and accurately identify any but fresh isolates. They probably could be maintained by frequent transfer and selection of only normal single-spore cultures each time stock cultures are transferred.

ENDOCONIDIOPHORA ON DOUGLAS-FIR

In a study of lumber-staining fungi on pine and hardwood lumber and logs in the Southeastern States in 1931 and 1932 (2), a very common species was found on hardwood. This species appeared morphologically similar to the European fungus *Endoconidiophora coerulescens* (5). It was later determined that the southern hardwood fungus was a distinct species and it was described as *E. virescens* (3). At that time it was pointed out that the European *E. coerulescens* was reported to occur only on pine and spruce, and it had a banana oil (amyl acetate) odor in culture besides showing slight morphological differences when cultures of it were compared with cultures of the southern hardwood fungus.

Infected sapwood of Douglas-fir lumber commonly shows a black surface growth and a gray staining of the wood below it. This stain is common on lumber in the central Rocky Mountain area, and probably in other areas. The lumber on which it has been observed was shipped from Oregon and infection is believed to have occurred in the woods or at the lumber mills. Infected lumber picked up on house construction jobs in Fort Collins, Colorado, harbored the fungus in viable condition (FIG. 2, A). While in Oregon in 1944, the writer visited a few Douglas-fir saw-

mills; he found the fungus at one mill in Portland and saw it in abundance on Douglas-fir slabs stacked along streets for fuelwood. Ernest Wright of the Division of Forest Pathology found it in check piles in stain control tests at Puyallup, Washington (FIG. 2, B).

The fungus mat on the surface of the wood consists of dense black mycelium and numerous black, hirsute perithecia. Where the mycelium is not too dense, the perithecia show up as small black tufts with a central neck or spine. Endoconidia are also present on the surface of infected wood but cannot be seen without

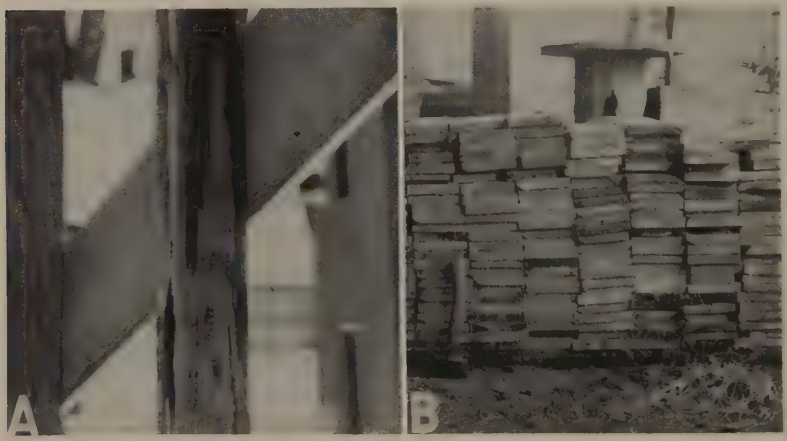

FIG. 2. *Endoconidiophora coerulescens* f. *douglasii* stain on Douglas-fir. A. On house framing timbers at Fort Collins, Colorado; B. on ends of untreated boards in stain control test pile at Puyallup, Washington. (B. was contributed by Ernest Wright.)

the aid of a good lens or dissecting microscope. The stain extends to the heartwood.

CULTURES OF ENDOCONIDIOPHORA. Success has been obtained in isolating the Douglas-fir staining fungus by collecting wood perithecia in good fruiting condition or by placing infected wood in a damp chamber until perithecia develop. Perithecia will usually develop on infected wood in 3 to 7 days after infection. When perithecia have matured, ascospores may be picked from the perithecial beaks with a sterile needle and transferred to cornmeal, malt, or potato dextrose agar media.

The fungus was obtained in pure culture in 1944 at Portland, Oregon. However, it did not grow well on the malt agar medium used and all cultures were dead on arrival at Beltsville, Maryland. The interval between the time of making the isolations and attempting transfers was 3 to 4 weeks. In 1945 the fungus was isolated at Beltsville from a short section of Douglas-fir "2 × 4" shipped from Portland, Oregon. These cultures also died after a short period, but during this time the fungus was compared with a culture of *Endoconidiophora coerulescens* from Europe. At a temperature of 22° to 25° C both fungi had the characteristic banana oil odor, but the European strain grew considerably faster than the Douglas-fir strain (47 mm. in 4 days and 81 mm. in 6 days for the European as compared to 11 mm. and 22 mm. for the American). The European culture would no longer produce perithecia and was somewhat faster growing than cultures described by Lagerberg *et al.* (4).

Later studies in a cooler laboratory at Fort Collins, Colorado, showed that the Douglas-fir fungus can be maintained satisfactorily in culture on any of the three culture media mentioned above. Growth has been much faster and more uniform than the earlier isolations indicated. It was not grown under constant temperature conditions. The temperature remained under 21° C and probably averaged between 16° to 18° C. Growth at this lower temperature was about the same as that recorded by Lagerberg *et al.* (4) for the European fungus, or about 100 mm. diameter in 10 days. Perithecia mature in culture in 7 to 12 days.

COMPARISON OF THE DOUGLAS-FIR FUNGUS WITH ENDOCONIDIOPHORA COERULESCENS. Morphologically, the *Endoconidiophora* on Douglas-fir is very similar to *E. coerulescens*. Perithecia are about the same in size, shape, length of neck, and in character of ostiole. Ascospores are 6–9 × 1.7–2 μ, which is about the same as given for the European fungus. Conidiophores are of two types, one tapering to a small diameter at the apex and the other enlarged to the apex. This character was not noticed in cultures of *E. coerulescens* from Europe, although conidia had about the same variability in size in both, with those from Douglas-fir ranging slightly smaller in diameter.

The hosts of these two fungi are different. In the United States the fungus has been found only on Douglas-fir. It has not been observed on spruce, hemlock, Sitka spruce, true firs, or on pine species in any areas where the writer has made observations. However, a sufficiently thorough search has not been made in some areas of the West to be sure it does not occur on some other host. On the basis of present information, it seems desirable to designate the western fungus a form of *E. coerulescens:*

Endoconidiophora coerulescens Münch forma **douglasii** f. nov.[3] (FIG. 3).

Perithecia atra, hirsuta, 160–190 μ lata, 180–210 μ alta, rostellis basi 30–40, apice 12–16 μ latis, 500–1000 μ longis, filamentis hyalinis 15–24 μ longis, basi 2 μ, ad apicem ad 1 μ attenuatis praeditis; ascosporae subcurvatae, hyalinae, 6–9 × 1.7–2 μ; endoconidiophora 50–250 μ longa, 5–10 μ crassa, alia apice attenuata, alia supra inflata; conidia 4–30 × 3–8 μ, hyalina, catenulata.

In lingo *Pseudotsugae taxifoliae* U. S.

Perithecia black, hirsute, 160–190 × 180–210 μ high, with necks 30–40 μ wide at base to 12–16 μ wide at tip and 500–1000 μ long, ostiolar filaments hyaline, 15–25 μ long, and tapering from about 2 μ at the base to 1 μ at tip; ascospores slightly curved, hyaline, 6–9 × 1.7–2 μ; endoconidiophores variable in length and thickness, 50–250 μ long by 5–10 μ thick, some tapering to smaller diameter at apex and others enlarged above; conidia variable in length and thickness, 4–30 × 3–8 μ, hyaline, in chains. Grows on cornmeal, malt, or potato dextrose agar media at temperatures slightly lower than ordinary room temperature of 25° C.

Collected on sapwood of *Pseudotsuga taxifolia* (Poir.) Britton in the western United States.

Type specimen (F. P. *70703*) deposited in the Mycological Collections, Bureau of Plant Industry, Beltsville, Md.

SINGLE-SPORE CULTURES. Twenty-three of 33 single conidial and single ascospore cultures developed fertile perithecia. Ten of the single-spore cultures developed no perithecia, either when grown separately or when grown together in all possible combinations. All ten nonperithecial cultures had perithecial primordia. Bakshi (1), who isolated *E. coerulescens* from pine wood in Eng-

[3] Latin diagnosis prepared by Edith K. Cash, Associate Mycologist, Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Maryland.

land, considered the species to be homothallic but some of his single-spore cultures did not develop perithecia.

An attempt was made to spermatize the 10 non-perithecial cultures by streaking spores and mycelium from a single-spore perithecial culture across 5-day-old petri-dish cultures. Fertile perithecia developed at or near the margins of the 5-day-old growth

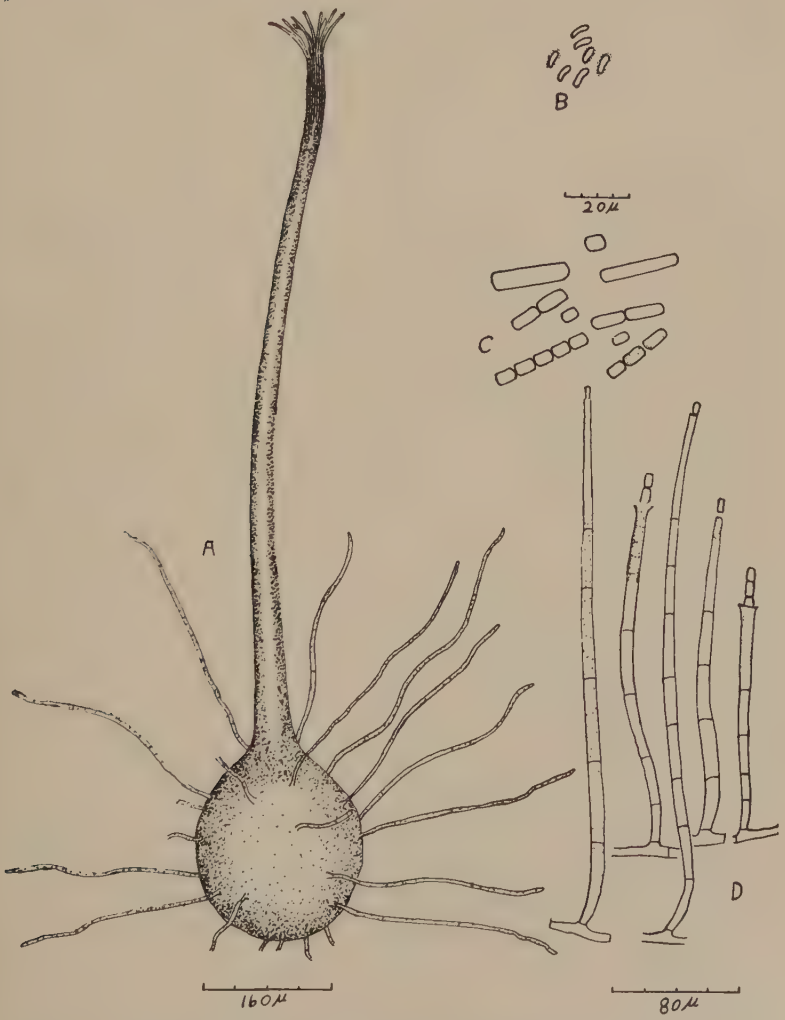

FIG. 3. *Endoconidiophora coerulescens* f. *douglasii*. A. Perithecium; B. ascospores; C. conidia; D. endoconidiophores with condidia.

in all 10 cultures in 6 days. These perithecia developed only in the area of the streaks. Cultures from fertile (single-spore) strains started at the same time required 8 days for the development of mature perithecia. This would seem to indicate either that some of the perithecial primordia that were in the proper stage of development were spermatized by the spores or mycelium from a normal perithecial culture, or that some combination of the two stimulated development of perithecia of the fertile strain more quickly than occurs under normal conditions.

SUMMARY

Two species of the *Ophiostomataceae* group of wood-staining fungi are reported on sapwood of coniferous lumber in the western United States. A species very similar to *Endoconidiophora coerulescens* but apparently confined to Douglas-fir is described as a form of the European species. It covers the sapwood of Douglas-fir with a black surface growth of mycelium, and stains the wood likht to dark gray. *Ophiostoma piceae*, another well-known European species, is also common on Douglas-fir lumber and numerous other coniferous woods in the West. It does not cause a pronounced stain but its *Graphium* fruiting stage is conspicuous on the wood.

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ON HYALODOTHIS CARICIS

William W. Diehl

(with 4 figures and map)

Among the many puzzling examples of immature and generally unrecognizable fungi that for some thirty years have come to my hand for determination there is a small number of specimens containing inflorescences of various species of *Carex* bearing similar, immature stromata that at each encounter I have hesitantly labeled "*Hyalodothis* (?) *caricis* Pat. & Har." because no other name seemed to apply. It is always embarrassing to assign a name to an immature fungus, and in these cases especially so because they were mostly found at points many thousands of miles apart along the northern and western periphery of the Pacific Ocean.

The species was questioningly described from immature stromata by Patouillard and Hariot in 1893 (Bull. Soc. Myc. Fr. **9**: 210–211) and is known only from their record of the two specimens found in 1889 by the Abbé Urbain Faurie on the Japanese Island of Kunashiri. The type material (Fig. 1) among Patouillard's specimens at the Farlow Herbarium of Harvard University, examined through the courtesy of the late Dr. David Linder, was found to resemble closely the specimens that are thought to be conspecific with it. Insofar as immature stromata are significant, they could all be representative of the same species. The black subspherical stromata, which suggest small shoe-buttons, arise upon immature florets and in some cases entirely enclose them. They show no hypothallus and, since their superficial conformation and habit suggest a member of the Balansieae, they might be referable to *Balansiopsis* were there characteristic clavicipitaceous perithecial walls in evidence. This lack of a hypothallus should serve to preclude confusing this species with the cariciphilous Indian *Ophiodothis caricis* Berk. & Curt. because, as shown by Atkinson (Jour. Mycol. **11**: 248–267. 1905), that species pos-

sesses a hypothallus, which he termed the "sclerotium." But without more matured frutifications to interpret its taxonomic relations *H. caricis* remains a taxonomic enigma.

There appears to be no subsequent report of the species unless, as seems likely, the two inadequate records of *Balansia vorax* by K. Togashi (Japan Jour. Bot. 2: 80. 1924) on *C. sachalinensis* from the Island of Rebun and by K. Togashi and F. Onuma (Bull. Imp. Coll. Agr. & For. Morioka **17**: 10. 1934) on *C. morrowi* from Iwate Province in northern Japan may refer to it. No Japanese specimens other than Faurie's have been available for examination but the comparable specimens now available from other Pacific areas are as follows:

In the Farlow Herbarium there is a fragment of an immature specimen labeled "*Ophiodothis vorax* on *Carex buxbaumii*" found at Sitka, Alaska in 1867 by J. T. Rothrock which had been turned over to W. G. Farlow from the Gray Herbarium. This specimen seems to have escaped any published record although Rothrock in his "Sketch of the Flora of Alaska" (Rept. Smithsonian Inst. **1867**: 57. 1868 [1872]) records *C. buxbaumii* from Sitka.

At Sitka J. P. Anderson in 1916 also found similar but smaller stromata on a different sedge, *C. pauciflora*.

In 1939 W. J. Eyerdam at Knight Island on Prince William Sound in Alaska obtained analogous immature stromata in two different gatherings of *C. limosa*. But in these instances the stromata, instead of being few and occasional on the inflorescences, were so numerous and so strikingly aggregated as to give each inflorescence of the host the appearance of a small blackberry (FIGS. 2, 3). A comparable blackberry-like mass of stromata is additionally available on a single spike of *C. enanderi* found in 1945 on the Island of Attu by G. W. Soule.

In 1943 Capt. G. A. Ammann on the Island of Kiska made two gatherings of similar immatured stromata which are possibly less crowded (FIG. 4), thus more like the type, among the florets of

FIGS. 1–4. *Hyalodothis caricis*. 1. Type material, × approx. 1, at Farlow Herbarium. Photo courtesy David Lindner. 2. On *C. limosa* W. J. Eyerdam no. *3232*, × 1. Photo by R. L. Taylor. 3. On *C. limosa*, × ½, showing habit. Photo by R. L. Taylor. 4. On *C. lyngbyei*, G. A. Ammann, × 1. Photo by R. L. Taylor.

FIGS. 1–4.

TABLE SUMMARIZING RECORDS OF *H. caricis* INDICATED ON MAP

Station	Lat.	Host	Date	Collector	Previous record in literature	Herbaria where deposited	Host determined or verified by:
+Japan-Isl. Kunashiri	48° N	*Carex* sp.	1889	U. Faurie	Patouillard & Hariot 1889	Farlow Herbarium	U. Faurie—?
−Japan-Isl. Rebun	40° N	*C. sachalinensis* Kuek.	1922	T. Togashi No. 18	Togashi 1924	?	Togashi—?
−Japan-Pref. Iwate	40° N	*C. morrowi* Boott	1928	F. Onuma No. 29	Togashi & Onuma 1934	?	Togashi & Onuma —?
+Alaska-[Sitka?]	57° N	*C. buxbaumii* Wahl	1867	J. T. Rothrock	0	Farlow Herbarium	Asa Gray—?
+Alaska-Sitka	57° N	*C. pauciflora* Lightf.	1916	J. P. Anderson No. 348	0	U. S. Myc. Collections, Beltsville, Md.	J. P. Anderson—?
+Alaska-Knight Isl.	61° N	*C. limosa* L.	1939	W. J. Eyerdam	0	U. S. Myc. Collections, Beltsville, Md.	F. J. Hermann
+Alaska-Kiska	52° N	*C. lyngbyei* Hornem.	1943	G. A. Ammann Nos. 20, 111	0	do & Herb. Univ., Mich.	F. J. Hermann
+Alaska-Attu	53° N	*C. enanderi* Hulten	1945	G. W. Soule No. 541	0	U. S. Myc. Collections, Beltsville, Md.	F. J. Hermann
+New Guinea	7° S	*C.* sp.	1939	M. S. Clemens No. 9567	0	U. S. Myc. Collections, Beltsville, Md.	F. J. Hermann
+New Guinea	7° S	*C. rara* Boott	1939	M. S. Clemens no No.	0	U. S. Myc. Collections, Beltsville, Md.	F. J. Hermann

+ = specimens examined; − = specimens not seen.

C. lyngbyei. Ammann's specimens have been made available through the courtesy of Dr. E. B. Mains of the University of Michigan.

Two specimens from New Guinea found by Mrs. M. S. Clemens in 1939 at altitudes between 7000 and 10,000 feet are similar although they are slightly more mature than the northern specimens noted above; they bear immature asci in locules without as yet the development of the perithecial walls characteristic of the Clavicipitaceae; one of the New Guinea specimens is on an undetermined species of *Carex,* the other on *C. rara.*

Although the blackberry-like masses of stromata in three of the above-cited specimens present a superficial aspect very slightly different from the others the characters of the several stromata are not significantly different. The high degree of aggregation in these instances may merely be indicative of a differental susceptibility reaction in these particular hosts rather than of a taxonomic difference in the fungus. The stromata are easily broken from their points of attachment and if subjected to the pelting of rain or snow in the winter season would doubtless be detached and fall to the ground. The peculiar and consistently immature state of the stromata, according to the seasonal lateness of dates of collection of the northern specimens, would have precluded their further maturity during the current season. It would thus seem that matured stromata would be found during the second season upon the ground rather than attached to inflorescences. The same effect could likewise be expected for those stromata produced at higher elevations farther to the south.

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A NEW SPECIES OF MONOBLEPHARELLA [1]

(WITH 1 FIGURE)

FREDERICK K. SPARROW [2]

The genus *Monoblepharella,* consisting of four species, is a small but interesting group of Phycomycetes by reason of the fact that the fertilized egg is propelled by the flagellum of the male gamete (Sparrow **1940**; Shanor **1942**; Springer **1945**). It has assumed further interest with the discovery by Johns (**1952**) that members of the well-known genus *Gonapodya* possess an essentially similar type of sexual reproduction.

The purpose of the present paper is to report a species of *Monoblepharella* which is distinct in certain essential features from previously described ones, namely, in the apparent lack of antheridia (and antherozoids), and in the formation of apogamous oospores which remain within the oogonium. It was isolated from soil collected in a roadside ditch in the immediate vicinity of Clarksdale, Mississippi, February 16, 1949.

The mycelium was of the delicate, flexuous, pearly gray type described for other species of *Monoblepharella.* Although most species of the genus possess irregularities of the mycelium, the present fungus was noteworthy in this respect. Numerous, up to 9 μ in diameter, catenulate expansions of the hyphae were formed which in some instances strikingly resembled those found in *Gonapodya.* Further resemblance to the latter genus was found in the frequent emergence from the distal catenulation of two diverging hyphal elements. The contents of the hyphae were disposed in the usual reticulate fashion save in actively growing apices which were homogeneous.

Zoosporangia were abundantly formed at about 24.5° C. The precise temperature relationship has not, however, as yet been

[1] Paper no. 978, Department of Botany, University of Michigan.

[2] Acknowledgment is made to the Executive Board of the Horace H. Rackham School of Graduate Studies for a grant which made possible the collection of this and other fungi in the Gulf States.

ascertained. These sporangia were of the usual siliquaeform type and were 34–47 μ long by 5–8 μ in diameter. They were borne either singly or in pairs at the tips of hyphae. After sympodial branching of the hyphae they appeared lateral, often in clusters (FIG. 1, a, b). The zoospores were ovoid to subcylindrical when swimming, 8 μ long by 5 μ wide, and possessed the usual monoblepharid internal structure and posterior flagellum (FIG. 1, b).

Oogonia were abundantly formed when water cultures were left undisturbed for several days at 30–31° C. They were obpyriform with rounded apex, narrow cylindrical base, and were 10–15 μ long by 7.5–8.75 μ in diameter, tapering to 2.5 μ at the base. Like other species of the genus, they contained in most instances (FIG. 1, e, compared with FIG. 1, f) a single egg bearing numerous large refractive globules. These oogonia were at first terminal (FIG. 1, g) but by sympodial branching of the hyphae often become lateral and occasionally clustered (FIG. 1, c, d, f, h).

No evidence has been found as yet for the existence of antheridia and antherozoids. This feature has been considered in some detail. Pure cultures on agar were obtained from which transfers were made to sterile soil water cultures baited with hempseed. Under these conditions an abundance of oogonia (and oospores) were formed, but so far as could be ascertained, no antheridia or other reproductive structures of any kind. Needless to say, this aspect of the fungus is still under investigation.

Under the aforementioned conditions oospores matured in profusion and these were uniformly endogenous. The latter fact was in agreement with observations in the original gross cultures and seemed to indicate that, typically, the oospores were endogenous and not so because of fouled environmental circumstances. Further work on the morphology and studies of the physiology of the fungus are being undertaken.

Since by reason of the aforementioned features the fungus is very distinct from other species of *Monoblepharella,* in which genus it undoubtedly belongs, it is described as new.

Monoblepharella endogena n. sp.

Mycelium amplum, hyphis tenuibus, flexuosis, ramosis, 2.5–6 μ dia. intus reticulate vacuolatis, plerumque multis irregularibus tumoribus ad 9 μ dia.

FIG. 1, a–h. *Monoblepharella endogena*. All × 970. a. Cluster of sporangia, two empty, a third developing. b. Zoosporangium discharging its zoospores. c, d. Mature endogenous oospores on hyphae developing new oogonia. e. Oogonium with four eggs. f. Cluster of mature oospores with new oogonia forming sympodially. g. Terminal oospore. h. Terminal oospore which by sympodial branching and subsequent oogonial formation of its supporting hypha appears lateral.

Sporangia siliquiformia, 34–47 μ longa, 5–8 μ dia., apices hypharum terminantia vel singula vel bina vel post hyphae ramificationem sympodialem quasilateralia. Zoosporis ovoideis vel subcylindricis, 8 μ longis, 5 μ latis postice cilio quam soma duplo vel triplo longiori praeditis. Oogonium primum terminale vel post ramificationem hyphae sympodialem saepe quasilaterale, clavatum vel obpyriforme, apice rotundatum basi anguste cylindricum, 10–15 μ longum, 7.5–8.75 μ crassum basi ad diametrum 2.5 μ angustatum, maturitate ovo singulo (rare ad quatuor) magno, refractivis globulis praedito, incluso. Antheridium ignotum. Oospora endogena, spherica, 7.5–10μ dia., etiamque guttulata membrana paulum incrassata pallide brunnea laevia.

In humo, per *Cannabis* semen illecebram culta, coll. F. K. Sparrow, Clarksdale, Mississippi, Feb. 16, 1949.

Mycelium well developed, of tenuous flexuous branched hyphae 2.5–6 μ in diameter, with many irregular swellings up to 9 μ in diameter, contents reticulately vacuolate. Sporangia siliquaeform, 34–47 μ long by 5–8 μ in diameter, occurring singly or in pairs at tips of the hyphae or after sympodial branching appearing lateral; zoospores ovoid or subcylindrical, 8 μ long by 5 μ wide, the posterior flagellum 2–3 times the length of the body. Oogonium at first terminal or after sympodial branching of hypha appearing lateral; clavate or obpyriform with rounded apex and narrow cylindrical base, 10–15 μ long by 7.5–8.75 μ wide, tapering to 2.5 μ at base, the contents at maturity forming one, rarely up to 4 eggs bearing numerous large refractive globules. Antheridium and antherozoids unknown. Oospore spherical, 7.5–10 μ in diameter, endogenous, with a slightly thickened light-brown smooth wall, contents bearing globules.

In soil, cultivated on hempseed. Clarksdale, Mississippi, collected February 16, 1949, F. K. Sparrow.

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SOIL AS A STORAGE MEDIUM FOR FUNGI [1]

ALEXANDER BAKERSPIGEL

(WITH 1 FIGURE)

Since 1918 a number of workers (3, 15, 16) have stored fungi for months or even years upon sterilized soil. Thus, in 1934 Green and Fred (10) preserved *Penicillium chrysogenum* Thom. and *Aspergillus* ssp. for over 2 years in soil. They found that after this time the fungi were viable and their colony characters were less changed than the control cultures growing on nutrient agars. Similarly from 1945–52, Miller (11, 12, 13), Miller *et al.* (14), Cormack (6) and Gordon (9) found soil to have a preservative and stabilizing effect upon Fusarium ssp.

In 1948, Ciferri and Redaelli (5) inoculated 3 species of dermatophytes on soil and preserved them for 3 years observing little pleomorphic change.

Dowding (7) and Emmons (8) have grown systemic fungi on soil. In 1951, Ajello and Zeidberg (1) and Emmons (8) isolated *Histoplasma capsulatum* Darling and *Allescheria Boydii* Shear from soil in nature. However as far as the writer is aware the systemic fungi have not been stored in the laboratory in this medium.

The following investigation, as well as determining the viability of additional species of fungi upon soil, compares the advantages of loam with clay, peat, sand and other natural materials, records whether or not changes in colony characters occur during storage, and measures the amount of increase of the inoculum.

[1] Contribution from the Department of Botany, McMaster University, and the Provincial Laboratory of Public Health, University of Alberta.

MATERIALS AND METHODS

MATERIALS. 18 fungi were used for the storage experiments. They were members of the Fungi Imperfecti, chiefly plant and animal pathogens. The species and their sources are as follows.

Group A

1. *Phoma* sp. from chrysanthemum, Ontario; 2. *Hormodendrum* sp.*a* soil, Ontario; 3. *Fusarium* sp., muskmelon, Ontario. 4. *Hormodendrum* sp.*b*, *Dracaena*, Ontario; 5. *Phyllosticta* sp., *Dracaena*, Ontario.

Group B

Dermatophytes: 1. *Trichophyton mentagrophytes* (Robin) Blanchard from patient 1453, Alberta; 2. *T. tonsurans* Malmsten and 3. *T. sulfureum* Sabouraud, both from patients in Ontario; 4. *Epidermophyton floccosum* (Harz) Langeron and Milochevitch, from patient 2053, Alberta; 5. *Microsporum audouini* Gruby, patient 2792, Alberta; 6. *M. canis* Bodin, patient 2794, Alberta; 7. *M. gypseum* (Bodin) Guiart and Grigorakis, patient, Ontario.

Systemic Fungi: 1. *Candida albicans* (Robin) Berkhout from patient 1891, Alberta; 2. *Sporotrichum Schenkii* (Hektoen and Perkins) Matruchot, patient, Michigan; 3. *Blastomyces dermatitidis* Gilchrist and Stokes, dog, Iowa; 4. *Histoplasma capsulatum* Darling, the U. S. Department of Health Culture Collection; 5. *Haplosporangium parvum* Emmons, deermouse lung, Alberta; 6. *Nocardia asteroides* (Eppinger) Blanchard, a patient, Michigan.

The storage materials for group A were loam *a*, sand, clay, peat and other natural materials whose origin and physical properties are shown in TABLE I.

The sole storage material for group B was loam *b*.

INOCULATION PROCEDURE. For group A, 135 cc. of the 7 storage materials (listed in TABLES II and III) were distributed into duplicate sets of 250 cc. Ehrlenmeyer flasks. For group B, 135 cc. of loam *b* alone were distributed into flasks. Distilled water equal to 25% of the water-holding capacities of the materials used was poured into each flask. The flasks were plugged with non-absorbent cotton and then autoclaved once at 15 lbs. pressure for

TABLE I

STORAGE MATERIALS AND SOME OF THEIR PHYSICAL PROPERTIES

Materials	Source	Weight of 135 cc. oven-dried material in gms.	Water holding capacity per 100 cc.	Density	pH
Loam *a*	Royal Botanical Gardens, Ontario	121.5	32	0.90	6.8
Loam *b*	Plant Science Dep't, University of Alberta	154.0	47	1.25	6.1
Clay	McMaster University, Ontario	183.5	22	1.36	7.0
Oak Sawdust	McMaster University, Ontario	15.5	35	0.12	6.9
Humified Peat	McMaster University, Ontario	36.5	94	0.27	6.5
Unhumified Peat	McMaster University, Ontario	9.5	191	0.07	6.7
Banding Sand	Central Scientific Co., Ontario	236.5	31	1.75	6.8
Kieselguhr	Peller Brewing Co., Ontario	16.2	115	0.12	7.2

20 minutes, which proved sufficient to prevent the growth of contaminants.

The 18 fungi were grown on agar slants from 1 to 3 weeks. Sufficient sterilized distilled water was added to obtain conidial suspensions. These were diluted to reach volumes equal to 25% of the water-holding capacity of the storage material to which they were added.

TABLE II

AVERAGE NUMBER OF VIABLE CELLS PER CC. STORAGE MATERIAL *

Fungus	Storage material	Age of sample (in months)									
		5	10	13	15	18	20	23	30	39	48
Phoma	Loam *a*	4900	4700	7800	5400	6500	7300	2000	2000	100	30
	Clay	6400	3000	2300	3300	2300	1500	0			
	Sawdust	600	100	0							
	Humified Peat	2800	400	900	100	800	0				
	Unhumified Peat	1700	100	100	0						
	Sand	800	0								
	Kieselguhr	1400	200	200	100	0					
Hormodendrum *a*	Loam *a*	2200	0								
	Clay	1100	0								
	Sawdust	0									
	Humified Peat	0									
	Unhumified Peat	0									
	Sand	100	0								
	Kieselguhr	300	0								
Fusarium	Loam *a*	7200	5000	7100	8400	8700	25000	10300	5000	100	80
	Clay	1500	300	400	900	1500	1000	0			
	Sawdust	6000	200	100	300	0					
	Humified Peat	2200	8400	8500	8800	8000	9000	2800	1500	1000	50
	Unhumified Peat	1300	2600	300	1200	1300	1000	0			
	Sand	100	0								
	Kieselguhr	2300	200	0							

* Approximate number of viable cells inoculated per cc. material was: for *Phoma* 104, *Hormodendrum a* 48 and *Fusarium* 44.

TABLE III

AVERAGE NUMBER OF VIABLE CELLS PER CC. STORAGE MATERIAL *

Fungus	Storage material	Age of sample (in months)							
		1	2	3	4	7	14	23	32
Phyllosticta	Loam *a*	132,000	375,000	520,000	600,000	290,000	450,000	60,000	85,000
	Clay	54,000	75,000	166,000	178,000	40,000	23,000	4,000	11,000
	Sawdust	5,000	39,000	36,000	17,000	12,000	9,000	2,200	1,000
	Humified Peat	58,000	27,000	43,000	22,000	7,000	0		
	Unhumified Peat	54,000	112,000	92,000	44,000	11,000	0		
	Sand	11,000	49,000	47,000	8,000	2,000	0		
	Kieselguhr	14,000	10,000	38,000	66,000	90,000	130,000	54,000	80,000
Hormodendrum b	Loam *a*	290,000	536,000	2,300,000	2,250,000	2,100,000	450,000	15,000	12,000
	Clay	256,000	326,000	1,100,000	965,000	995,000	200,000	53,000	1,000
	Sawdust	113,000	204,000	455,000	420,000	0			
	Humified Peat	361,000	154,000	100,000	200,000	0			
	Unhumified Peat	21,000	45,000	32,000	220,000	0			
	Sand	132,000	118,000	106,000	3,000	0			
	Kieselguhr	168,000	132,000	310,000	840,000	flask broken			

* Approximate number of viable cells inoculated per cc. material was: for *Phyllosticta* 1333 and *Hormodendrum b* 1207

As controls, the fungi were inoculated to fresh agar slants, group A on potato-dextrose-agar (Difco), group B on Sabouraud's medium in which phytone was substituted for peptone and yeast extract added. All of them were transferred to fresh agar each time the storage flasks were sampled.

The flasks containing the fungi of group A were stored at room temperature from 3 to 4 years, those containing group B, over a year.

In group A, when the flasks were about to be inoculated, the number of viable cells to be introduced was determined by the plate dilution method. For group B, no counts were made.

SAMPLING PROCEDURE. Group A: To test a sample, 10 cc. of storage material was measured from each flask into a sterilized graduated aluminum cup. It was transferred to the first of a dilution series of water blanks. The first blank was shaken 3–5 times to break up the material and then dispensed to the other blanks in the series, distributing the material by drawing it up and down several times with a sterile pipette. One milliliter of the final dilutions of the fungi was delivered to each of 6 plates. Potato-dextrose-agar was then added and the agar and inoculum rotated gently. Incubation was at 25° C for 5 to 10 days.

Group B: To test a sample, a small quantity of loam was removed by a sterilized platinum loop and streaked on the agar. Incubation was at 24° C for 1 to 3 weeks.

As can be seen from TABLES II and III, in group A, *Phoma* sp. and *Fusarium* sp. were sampled 10 times in a period of 4 years while *Hormodendrum* sp. *b*, and *Phyllosticta* sp. were sampled 8 times in a period of 2½ years. In group B, all the dermatophytes and systemic fungi were sampled 4 times during the year of storage.

TABLES II and III record the average number of colonies that developed on the plates.

CONDITION OF STORED FUNGI

VIABILITY. The results of the storage of group A upon 7 materials (TABLES II and III) show that the fungi remained viable from 1 to 4 years.

Loam is the most satisfactory material for storage. It is true

that one of the two Hormodendra eventually died upon it, but the large number of viable cells counted at the end of 5 months showed loam to be the most satisfactory medium for this fungus also. The two fungi that survived storage for 48 months (*Phoma* sp., *Fusarium* sp.) did so on loam.

Since loam *a* proved to be the best medium for group A, the 13 fungi of group B were all stored on loam. The source of the loam used for them (loam *b*) is shown in TABLE I. At the time of writing (13 months later) all these dermatophytes and systemic fungi are still viable on loam. In this respect therefore, loam *b* was superior to Sabouraud's agar on which one dermatophyte, *Epidermophyton floccosum,* did not survive longer than 3 months.

STABILITY. Of the 18 fungi growing on control agar-slants, a number of species became pleomorphic. Thus, in group A, *Fusarium* produced great amounts of white hyphae which overgrew the original pink mycelium while *Phyllosticta* and *Phoma* produced at the center of the colony tufts of brownish aerial mycelium which extended peripherally. In group B, every one of the dermatophytes produced white tufts of aerial mycelium which overgrew the cultures so that they became indistinguishable one from another.

On the other hand when cultures were made from flasks of loam *a* and loam *b* on which the above fungi had been stored, the new mycelia were in the "wild" or "primary" state with no trace of pleomorphic growth on any of them.

FIG. 1 *a, c, e* shows in the left hand vertical column wild type cultures of *T. mentagrophytes, M. gypseum* and *Phoma* sp. These were obtained from loam *a* and *b* on which they had been stored from 13 to 48 months. The right hand vertical column shows in *b, d* and *f* the pleomorphic characters of these fungi after the same period of storage on the control agar slants.

INCREASE. In the first 1–2 months following inoculation, the storage material was moist and was covered and permeated by webs of mycelium and masses of spores. Later the soil dried up and showed no visible trace of fungus.

In *Phoma, Fusarium, Hormodendrum b,* and *Phyllosticta,* during the storage period, the viable cells per cc. of loam increased

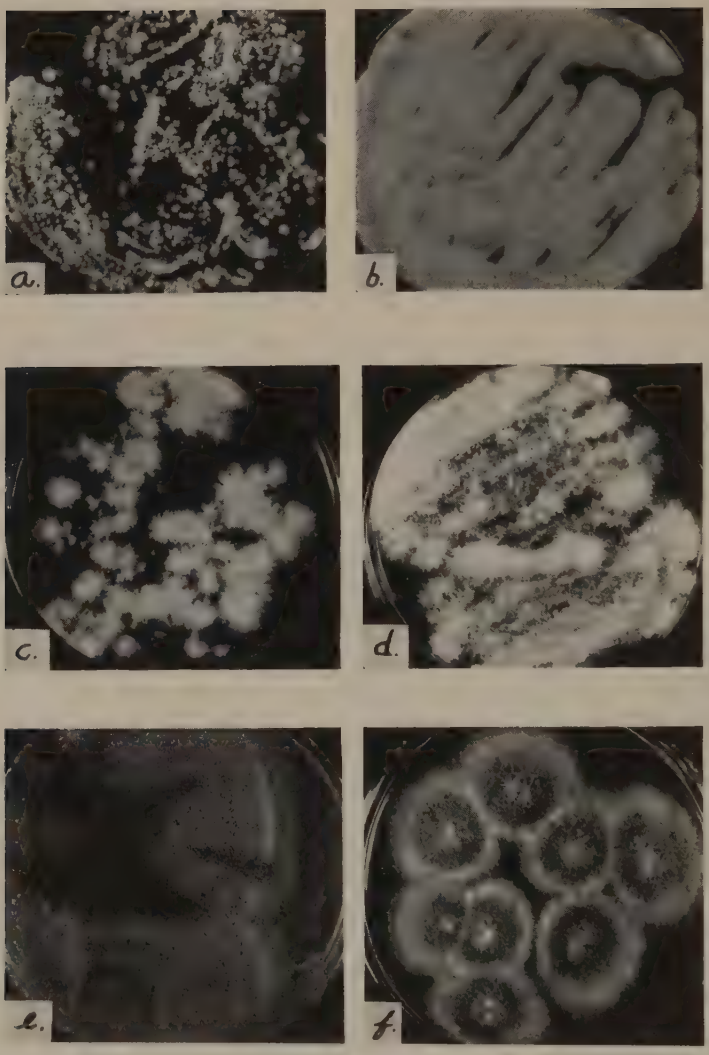

FIG. 1. *Wild* and *pleomorphic* cultures of fungi. Left, fungi after storage from 13 to 48 months on loam *a* and *b*. Right, controls maintained for the same time on agar. *a* and *b*, *Trichophyton mentagrophytes; c* and *d*, *Microsporum gypseum; e*, and *f*, *Phoma* sp. *a* and *b*, 12 day old cultures on Sabouraud's dextrose agar; *c*, 9 day; *d*, 11 day; *e* and *f*, 8 day. The cultures *b*, *d* and *f* are either completely pleomorphic or show centres and peripheries with tufts of mycelium. All photographs ½ natural size.

from 75 to 2000 fold. This increase went on for even 20 months after the material had become dry.

Explanations for this unexpected occurrence might be that, in the initial moist phase, clumps or chains of conidia were formed, then during prolonged storage, these clumps and chains of conidia (and mycelial strands also) broke up, causing an increase in the number of viable cells. The mixing of the contents of the flasks prior to sampling aided in this process. *Erysiphe polygoni* and *Piricularia oryzae* (**4**, **17**, **18**) are able to germinate under low relative humidities. Perhaps additional spores of our species of fungi germinated as the soil dried out, causing an increase in the number of viable cells.

ACKNOWLEDGMENTS

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SUMMARY

1. Of 18 species of fungi stored on loam, 17 remain viable for 1 year, and 2 for as long as 4 years.

2. All the fungi that were preserved in soil remained in the original wild or primary state, whereas on agar the dermatophytes, *Phoma* sp., *Phyllosticta* sp. and *Fusarium* sp. became pleomorphic.

3. In loam, the cells of *Phoma, Fusarium, Phyllosticta* and *Hormodendrum b* increased throughout the medium.

4. This investigation suggests that soil storage of fungi in the laboratory would obviate the laborious periodic transfer of agar stock-cultures and would provide a rich source that may be drawn from repeatedly for years. Most important, it would prevent pleomorphic changes in the fungi.

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W. LAWRENCE WHITE

R. G. H. Siu and E. T. Reese

(with portrait)

Doctor W. Lawrence White, Director of the Farlow Library and Herbarium and Associate Professor of Botany, Harvard University, died on July 30, 1952 at the age of 44. With his passing the mycological world lost one of its leading and potentially dominant taxonomists and stimulating personalities. Recognized among mycologists for his pioneering work in several areas of taxonomic mycology he was known even more widely for his reputation as an authority on Tropical Deterioration by the military, industrial and scientific world. With his appointment in 1948 as the new Director of the Farlow Herbarium he soon renovated the organization which had lain fallow since the passing of Dr. David Linder. Clearly a renaissance was in progress. Prospects for the maturation and harvest of his own research efforts and for the future of the Farlow could not have been brighter. But the anticipation was not to be fulfilled. All was obliterated in a split-second by a dreadful automobile accident on Concord Pike on rainy July 27, 1952.

Dr. White was born in Salina, Pennsylvania, on May 29, 1908. His undergraduate days were spent at the Pennsylvania State College. There he was deeply influenced by Professor L. O. Overholts, whom he served as laboratory assistant. They had much in common—farm background, modesty, friendliness, sincerity, ability and, above all, an intense devotion to taxonomy. A strong bond of affection naturally developed. Many of the professor's mannerisms and work habits were assiduously copied by the young student. One of these, which was to serve Dr. White well later in life, was the almost fanatical accumulation of mycological facts and tidbits for future reference. Under the steady guidance of Professor Overholts, White gained an early intimacy

with the Basidiomycetes both in the field and the laboratory. His knowledge of the Polyporaceae and the Thelephoraceae was supplemented by the acquisition of a good understanding of the rusts from Professors F. D. Kern and H. W. Thurston, and of the smuts from Professor G. L. Zundel. It was unusual that an undergraduate was provided such excellent and intensive mycological guidance. Even more important to White was the friendly interest shown him by these professors, and by Dr. J. W. Sinden. The latter was largely responsible for his going to Cornell after receiving the baccalaureate degree in 1934.

A different directional influence was exerted during his graduate study at Cornell University. Laboratory emphasis replaced field trips. From his major professor, Dr. H. M. Fitzpatrick, he learned to channel his propensity for collecting tidbits into logical order and coherent taxonomic patterns. Through Professor H. H. Whetzel he developed an enthusiastic interest in the inoperculate discomycetes. For his own specialization Dr. White selected the large and complex group of the Helotiaceae. He plunged into the examination of the group with such skill and intensity that within five years he was thoroughly familiar with these fungi. Unfortunately, economic pressure forced Dr. White to accept more remunerative employment as soon as he completed his doctorate in 1940. While several papers covering small segments of this research did reach publication, a monograph was never put into final shape. Its completion would have been a significant contribution to mycology; and he was the one best qualified to execute it. During the later years, he frequently talked about the manuscript with deep-felt nostalgia. There was, at times, a suggestive twinkle in his eyes—possibly some of his future graduate students would complete the work he had started. Had fate been generous with the years this might have come to pass. His graduate years were interspersed with seasonal employment, which rounded out his mycological training. Two summers were spent studying Ascomycetes with Dr. F. J. Seaver at the New York Botanical Garden (1936, 1937). Two more were spent working on the Dutch Elm disease.

Having familiarized himself with Basidiomycetes and Ascomy-

cetes, Dr. White selected as his first post-Ph.D. professional employment, one which provided him an excellent opportunity of extending his knowledge into the Fungi Imperfecti. In 1941 he became Bibliographer and Assistant Curator of Fungi at the Farlow Herbarium under the late Dr. D. H. Linder. His principal energy was directed towards bringing Seymour's Host Index up to date. This particularly painstaking revision was completed in manuscript form up to 1942.

Dr. W. L. White examining mildewed fabric at the Quartermaster Tropical Deterioration Research Laboratory. 1947.

During this period he met Mary Rebecca Old, a graduate student in Biology at Radcliffe, and they were married in October 1942. "Becky" continued to work as an assistant in research to aid financially during the first years of their marriage.

With the inception of World War II the attention of scientific, industrial and military minds became sharply focused upon the

problems of tropical deterioration. What had been anticipated as a brilliant future in classical taxonomic mycology was diverted to mycological research in connection with the mildew-proofing of materiel with the Chemical Corps at Massachusetts Institute of Technology in 1943.

This was his first exposure to non-academic scientific life. The previous limitation of his activities to highly specialized taxonomic research revealed its eminent disadvantages in the strange environment. His approach and his personality did not fit into the alien schema of a highly practical, management-conscious organization. The demands for concurrent participation in multifold activities involving conflicting codes of behavior, rapid decisions based on approximations, mental resiliency and adaptations under compromising situations, impinged on unsuspecting Dr. White. His educational foundation provided neither the requisite points of departure, nor behavior models, nor "types" to which he could refer. Accordingly, when he went into military research with the Host Index unpublished, he was unable to carry on both activities at the same time. He could not switch back and forth from the immediate practical approximations of war-time deterioration problems to the detailed, meticulously accurate, patient completion of the Host Index. His compromise was to drop the Host Index. Viewed scientifically, this was unfortunate. Viewed practically, what other course was open to him?

Similar bifurcations confronted Dr. White when he became Chief of the Tropical Deterioration Research Laboratory in July 1944—a post he held for 4 years. Should he devote his energies to fundamental taxonomic research on the microorganisms themselves, or should he become involved in the practical and managerial demands of the situation? Recognizing his principal talents and envisioning that the war would soon be over, he wisely chose the basic research avenue. He adopted the singular purpose of setting up the Quartermaster Culture Collection on a firm taxonomic basis. The managerial, administrative and practical unpleasantries he would ignore and stoically bear the consequences. That his intuitive judgment was wise no one would deny today. The Collection soon became the focal point for much

and vigorous world-wide basic research in taxonomy, physiology and biochemistry. The cumulated data serve today as the basis for the practical development of mildew-proofing treatments for textiles, leather, paper, wood and other materials. Its cultures are the commercial standards used in annual procurement of millions of dollars worth of fungus-proofed materiel.

The four years exposure to this highly practical environment was not without impression upon his general outlook and thinking. His first organizational actions at the Farlow Herbarium and his later philosophy of taxonomic research provide an indication of this influence.

When Dr. White became Director of the Farlow Herbarium in April 1948, he was faced with the necessity of operating on funds the purchasing value of which had undergone a drastic decline. One of his first actions was to change the time-honored system of filing specimens. The phylogenetic or natural scheme was replaced by an alphabetical system, based on large groups. The distribution of specimens by mail was restricted. Boxes of unclassified material of unknown value were discarded. Only by such drastic actions was he able to hew out a bit of time for his own research.

During the war, Dr. White was deeply impressed with the eternal and awesome consequences of mistakes—mistakes that set up chains of irreversible decisions, acts and consequences. Corrections never seemed to catch up. This recognition moved him to adopt extreme caution in the field of his own specialty. His recent publications reveal a bringing together of information on organisms, specimens and records dealing with a particular species. But he rarely committed himself on the validity of names. He was never certain that he had *all* the evidence. As a specialist in several groups, he recognized the many misidentifications cluttering the literature. He hesitated to add another. As a mycologist of wide acquaintance, he was familiar with the abilities of his contemporaries. He knew that the reports of some were based on inadequate study and hasty publication. Wasn't it logical to assume that some of the "authorities" of the past were equally negligent? He viewed the straightening out of mycological tax-

onomy as a hopeless task, unless drastic action were taken. First, a halt must be called to the introduction of new names. Second, there must be a vigorous reworking of the entire taxonomic literature, and a weeding out of errors. He determined to set an example. One may demur that if the most skilful experts—and Dr. White certainly was a prominent one—do not set forth their views and estimates on new species, from whom will the less-informed learn? Yet Dr. White felt that drastic ailments require drastic remedies. He was willing to sacrifice his own publications for the cause.

Recognition came to Dr. White early. He served as vice president of the Mycological Society of America, and as Secretary and Chairman of the Microbiological Section of the Botanical Society of America. While with the Quartermaster Laboratory, he was invited by the Air Force to serve as mycologist on the round-the-world flight of the Air Technical Service Command Tropical Science Mission. The field experience gave him valuable first hand information on the extent and nature of deterioration of materiel by microorganisms. Recognition of White as the authority in this field is attested to by the fact that at the Farlow he was working on two projects for governmental agencies (Chemical Corps; Quartermaster Corps), and his assistance was being sought by three others.

This sketch brings us to early July 1952. Here was a most competent taxonomist on the threshold of his most productive period. Here was a world authority on the Helotiaceae and the Fungi Imperfecti, with a wealth of experience in the Basidiomycetes and Ascomycetes. Here was a friendly, democratic, helpful and competent professor. Here was a rare combination of the scientific and the practical, the academic and the managerial. Here was the synthesis of an essence to be handed down to the first of many graduate students and to be distilled for the scientific world. Then fate stepped in.

We who called him friend are thankful that his path crossed ours. His association has enriched our lives. His memory we cherish. We recall fondly the many incidents typifying the man —how he pounded his hand through a streetcar door when the

driver failed to stop—how his taste in food went to hamburger and potatoes—how he loved to stay up all night in discussion groups loudly putting forth his opinions. We remember that his struggle for an education was hard, that his success was largely due to his inherent goodness, his stubborn persistence, his intuitive taxonomic sense, and to those who loved him and helped him on. He needed no hobby; his enthusiasm and enjoyment were in his work and his family.

PIONEERING RESEARCH LABORATORIES,
U. S. ARMY QUARTERMASTER CORPS,
PHILADELPHIA, PENNSYLVANIA

PUBLICATIONS OF W. LAWRENCE WHITE

(arranged chronologically)

1. (with H. W. Thurston, Jr.). Notes on the Rusts of Pennsylvania. Proc. Penna. Acad. Sci. **7**: 140–148. 1933.
2. A new species of *Chondropodium* on *Pseudotsuga taxifolia*. Mycologia **28**: 433–438. 1936.
3. Note on *Conidiobolus*. Mycologia **29**: 148–149. 1937.
4. (with H. H. Whetzel). Pleomorphic life cycles in a new genus of the Helotiaceae. Mycologia **30**: 187–203. 1938.
5. (with H. H. Whetzel). *Mollisia tetrica, Peziza Sejournei,* and the genera *Phaeociboria* and *Pycnopeziza*. Mycologia **32**: 609–620. 1940.
6. A Monograph of the genus *Rutstroemia* (Discomycetes). Lloydia **4**: 153–240. 1541. [Thesis-Cornell.]
7. A new Hemiascomycete. Canadian Jour. Res. **20(C)**: 389–395. 1942.
8. Studies in the genus *Helotium*. I. A review of the species described by Peck. Mycologia **34**: 154–179. 1942.
9. Studies in the genus *Helotium*. II. *Lachnum pygmaeum* and the status of the genus *Helolachnum*. Amer. Midl. Nat. **28**: 512–523. 1942.
10. Studies in the genus *Helotium*. III. History and diagnosis of certain European and North American foliicolous species. Farlowia **1**: 135–170. 1943.
11. Studies in the genus *Helotium*. IV. Some miscellaneous species. Farlowia **1**: 599–617. 1944.
12. (with R. Singer and W. H. Snell). The taxonomic position of *Polyporoletus sublividus*. Mycologia **37**: 124–128. 1945.
13. Deterioration of Quartermaster fabrics in the tropics. Quartermaster Rev. **26**: 16–17; 63–64; 67. 1946.
14. "Mycological factors," pp. 25–46 in Report of Army Air Forces Tropical Science Mission. Tropical Deterioration of Air Force Materiel and Equipment. Air Technical Service Command, Wright Field, Dayton, Ohio. 1946.

15. (with Mary H. Downing). The identity of *"Metarrhizium glutinosum."* Mycologia **39**: 546–555. 1947.
16. (with R. G. H. Siu). Resistance of resin-impregnated cotton fabrics to microorganisms. Ind. and Eng. Chem. **39**: 1628–1630. 1947.
17. (with R. T. Darby, Gladys M. Stechert, and Kathryn Sanderson). Assay of cellulolytic activity of fungi isolated from fabrics and related materials exposed in the tropics. Mycologia **40**: 34–84. 1948.
18. (with R. G. H. Siu and E. T. Reese). The black Aspergilli in relation to cellulosic substrata. Bull. Torrey Bot. Club **75**: 604–632. 1948.
19. (with C. C. Yeager and Helen Shotts). History, distribution and economic significance of the cellulose-destroying fungus *Memnoniella echinata*. Farlowia **3**: 399–423. 1949.
20. (with E. T. Reese, H. S. Levinson, and Mary H. Downing). Quartermaster Culture Collection. Farlowia **4**: 45–86. 1950.
21. (with G. R. Mandels and R. G. H. Siu). Fungi in relation to the degradation of woolen fabrics. Mycologia **42**: 199–223. 1950.
22. (with Mary H. Downing). *Coccospora agricola* Goddard, its specific status, relationships, and cellulolytic activity. Mycologia **43**: 645–657. 1951.
23. (with R. T. Darby). A refrigerator cabinet for fungal cultures. Mycologia **44**: 578–580. 1952.
24. (with Mary H. Downing). *Humicola grisea* Traaen, soil-inhabiting, cellulolytic Hyphomycete. Mycologia **45**. 1953 (in press).
25. (with G. D. Darker). Supplement to the Host Index of the Fungi of North America. (In manuscript; approx. 400 page book—unpublished.

Note: Government and University reports, abstracts, and book reviews have been omitted from the above list.

NOTES AND BRIEF ARTICLES

Synchytrium urticae

In a recent paper published shortly after his death Cook [1] described a new species of *Synchytrium* on *Urtica chamaedryoides* and named it *S. urticae*. This binomial, however, had been used previously by Sorokin [2] for a species which he found on *U. dioica* in Russia in 1873. It is surprising that Cook did not mention Sorokin's species because it was listed by Saccardo [3] and referred to as a questionable species by Tobler [4] in her monograph of *Synchytrium*.

Sorokin gave no measurements of his species but figured and described the resting spores as reniform, globose, oblong and elongate with a conspicuous pore and a densely verrucose or punctate, luminous, gray, fragile wall, as shown in the accompanying photograph of his drawings. According to his report the infected plants were recognizable from a distance by the yellow color of their leaves, which later dried up and shriveled. In the early stages of development of the parasite the infected epidermal cells do not differ from the neighboring healthy ones, but as the fungus matures the former cells become greatly enlarged. Nevertheless, they protrude only slightly above the surface of the adjacent epidermial cells, which apparently undergo little or no change. From Sorokin's figures it thus appears that his species produces only simple, unicellular galls. However, in herbarium material at the New York Botanical Garden which bears the name *S. urticae* and was distributed by Roumeguère (Fungi sel. exs. no. *1737*),

[1] Cook, M. T. 1952. Species of *Synchytrium* in Louisiana. VII. A new species on *Urtica chamaedryoides*. Mycologia **44**: 827–828.

[2] Sorokin, N. 1873. A few words about a new fungus, *Synchytrium urticae*. Contrib. 3rd Mtg. Russian Sci. Kiev, Bot. Trans., pp. 39–42, pl. 3. (Trans. from Russian.)

[3] Saccardo, P. A. 1888. Sylloge Fungorum **7**: 293.

[4] Tobler, G. 1912. Die Synchytrien. Studien zu einer Monographie der Gattung. Arch. protist'k. **28**: 141–238.

the author found numerous oval, 250 × 300 μ, elongate and almost spherical, 200–400 μ, composite, open and empty, protruding galls on the leaves of *Urtica dioica*.

Cook described his *S. urticae* as follows: "Galls abundant on stems, leaves, and flowers; more or less spherical, 48 × 48 to 93 × 156 μ. Sorus light yellow becoming dark yellow; 39 × 39 μ. Sporangia about 40 in number and 8–15 μ in diameter." Apparently no resting spores were found, and his observations concern only sporangial sori and sporangia.

However, in prepared slides of his species which Dr. Cook kindly sent me shortly before his death, the sori, sporangia and galls were found to be considerably larger than described by him. The sori were sub-spherical, 54.6–127.5 μ, or oval, 52–60 × 80–119 μ, and in several mature and ruptured ones 18 to 56 sub-spherical to spherical, 20–28 μ, oval, 17.4–20 × 34–43 μ, angular and polygonal sporangia were present. Galls were present on both sides of the leaves as well as on the stems, petioles and flower buds. They were composite, often compound, isolated or confluent, usually conspicuously protruding, predominantly sub-globular, hemispherical or sub-hemispherical, 180–244 × 300–720 μ, with an apical pore and a central canal extending down to the infected cell.

Whether or not Sorokin's and Cook's species are identical is open to question, but from Sorokin's description and figures they appear to be quite different. On the other hand, the galls on the material distributed by Roumeguère as *S. urticae* Sorokin on *U. dioica* are fundamentally similar to, but smaller than, those produced by Cook's species on *U. chamaedryoides*, and it is not improbable that Sorokin's brief description may be incomplete or incorrect. Equally plausible is the possibility that Roumeguère's material may relate to Cook's instead of to Sorokin's species. In this connection it is to be noted again that Sorokin found only resting spores, while Cook reported only sporangial sori and sporangia. Accordingly, the reported differences in gall structure and complexity produced by the two species may not preclude their being identical, because in some species, i.e., *S. cellulare*, the resting spores may produce simple, unicellular galls while the sporangial sori produce composite, multicellular galls on the same host. Ob-

viously, the problem of their identity cannot be solved until both species are fully known, but in the event Cook's species proves to be different and valid its name must be changed.—JOHN S. KARLING, Purdue University, Lafayette, Indiana.

Sorokin's plate of *Synchytrium urticae*. FIG. 1. Epidermal cells of *U. dioica* with a young parasite. FIG. 2. Mature *S. urticae*. FIG. 3. Profile of a cell with a resting spore of *S. urticae* n. sp. FIG. 4. Crushed spore of parasite. FIG. 5. Separated resting spores.

THE IDENTITY OF CORIOLOPSIS CROCATIFORMIS

In the examination of type material of polypores, it was found that some of the microscopic characters of *Coriolopsis crocatiformis* Murr. differed from the original description (N. Y. Bot. Gard. Bul. 8: 139. 1912). Since microscopic details are now considered to be essential for the diagnosis of a species, the type collection, obtained from the New York Botanical Garden, was carefully re-examined.

The macroscopic features are as originally described (FIG. 1, A). The microscopic characters which were given as "spores sub-

FIG. 1. *Coriolopsis crocatiformis* from type material. A. Upper surface and pore surface, × 1. B. Hymenium (much enlarged) showing the setae, basidia, spores and tramal hyphae. C. Cross section of the tubes showing the hymenium with numerous setae.

globose, smooth, hyaline, 3.5 μ; cystidia none" were not in entire agreement, for in 2 per cent KOH mounts it was found that the spores were cylindric-ellipsoid, 3.5–5 × 2–2.5 μ (FIG. 1, B), and that subulate setae 45–85 × 6–7 μ were present (FIG. 1, B, C). An emended description follows: Pileus sessile, spongy when dry, 1–2 × 1.5–4 × 0.2–0.5 cm.; surface yellowish-brown ("Ochraceous-Tawny" of Ridgway, "Color Standards and Color Nomenclature"), tomentose to spongy, velvety to touch, uneven, sulcate; margin thin, sterile below; context concolorous with the surface, soft and cottony, 0.1–0.2 cm. thick, the hyphae brown in KOH, thin- to thick-walled, occasionally septate, rarely branched, 2–4 μ in diame-

ter; tubes slightly darker than the context, 1–3 mm. long, the mouths irregular, becoming labyrinthiform by splitting of the walls of adjacent tubes, averaging 2 per mm., edges thin, somewhat lacerate to toothed, the tramal hyphae similar to the context hyphae except more frequently septated and branched; setae numerous, subulate, sometimes slightly enlarged at the base, thick-walled, 45–85 × 6–7 μ; basidia 9–14 × 4–5 μ; spores hyaline, smooth, apiculate, cylindric-ellipsoid, 3.5–5 × 2–2.5 μ.

The presence of setae relates this fungus to *C. sarcitiformis* Murr. which differs in having a radially fibrous upper surface, smaller pores, and shorter setae. *C. melleoflava* Murr. (= *Irpex flavus* Kl. according to Bresadola in Ann. Myc. **14**: 232. 1916) slightly resembles this fungus but differs in having abundant capitate cystidia. No other species are known which invite comparison.

This fungus is known only from Belleville, Oaxaca, Mexico, and grateful acknowledgment is made to the New York Botanical Garden for the loan of the type collection. Thanks are also due to Dr. J. L. Lowe under whose direction this work was done.— JOHN HUNT, State University of New York College of Forestry, Syracuse, N. Y.

PENICILLIUM WITHIN OVULES OF ZAMIA

In connection with studies on fertilization and embryo development in *Zamia* which have been conducted by one of the authors (G. S. B.), shipments of living carpellate cones collected in Florida have been received at the University of Wisconsin Botany Department at intervals through the early weeks of the summer during each of the past few years. Many of the cones, representing *Z. umbrosa* Small, were collected near Gainesville by Dr. George Weber of the University of Florida, but numerous cones of another species (*Z. floridana* A. DC.) were obtained from the vicinity of Miami and from Dunnellon.

When, in 1950, some of the *Z. umbrosa* cones were dissected and their ovules opened, it was observed that a green mold was present in the nucellar cap area above the gametophyte. In other

instances no mold was visible but, as in the case of the ovules showing fungal spores, the nucellus was shrunken and abnormalities were evident in the upper part of the gametophyte. The latter was commonly slightly discolored or translucent and the archegonia were empty. In some cases mold was observed to fill the empty archegonia. A few gametophytes were also seen in which the micropylar end was considerably distorted and no archegonia had been formed. The ovules, however, usually appeared normal from the outside; only occasionally could a faint browning be detected on or through the integument at about the level of the nucellar cap.

From several surface-sterilized ovules aseptically cut open lengthwise, spores were removed to agar plates, and the fungus was thus easily brought into pure culture. Several ovules which showed no spores when opened but which exhibited discoloration of the gametophyte were placed in sterile moist chambers. These always yielded a crop of spores in the nucellar cap region within two or three days, and a green line of spores often followed the nucellus around the upper half of the megagametophyte. Hence it was concluded that mycelium probably was present throughout this entire portion of the nucellar tissue at the time of removal of the ovule from the strobilus. Bits of nucellus aseptically removed from ovules showing internal discoloration but no spores, quickly yielded mycelium and conidia when placed on the surface of nutrient agar in petri dishes. However, in no case was a mold growth obtained when aseptically-removed bits of discolored gametophyte were plated on agar, and in only one instance in several trials was a fungus recovered from suspected integument tissue similarly treated.

During July, 1950, approximately fifty isolations of the *Zamia* ovule mold were made. The isolates came from ovules removed from several different *Z. umbrosa* cones from two or three separate collections—all from the Gainesville area. In the summer of 1952 additional isolates were obtained from perhaps a dozen ovules, including some from *Z. floridana* strobili collected in the vicinity of Dunnellon. The same fungus was isolated in every case. Although no isolations from material originating in southern Florida were attempted, ovules exhibiting pathological features identical

with those described above were encountered in the Miami collections; thus it seems probable that the same fungus was present here also.

Study of the *Zamia* mold in plate culture on various media indicated that it belonged to the Asymmetrica-Velutina group of *Penicillium,* but a definite species determination could not be made. Cultures were then sent to Dr. K. B. Raper, who kindly examined them and reported that the fungus did not fit well into any of the described species. He suggested, however, that it might be considered an aberrant member of the *P. citrinum* series. Tests in the writers' laboratories, both on agar and in shaken-flask liquid cultures, have made clear that none of a half dozen randomly-selected isolates produces citrinin. That, together with other considerations, has led to tentative assignment of the fungus to a position near *P. corylophilum.*

Insofar as the authors can discover, this is the first report of the occurrence of *Penicillium* within the ovules of *Zamia;* but the frequency with which the organism was encountered in cones received from Florida during the past three years suggests that invasion of ovules must be common in that geographical area. Since the fungus usually is found in ovules showing no external damage and since the micropyle is sealed early in the development of the ovule, it would appear that the organism must gain entrance at the time of pollination. The fungus seems to show a strong preference for nucellar tissue and there is no evidence that it enters the gametophyte except to grow down into aborted archegonia. The observed modification of gametophyte tissue may result from diffusion of toxic material formed in the invaded nucellar cap area. The observations made suggest that the organism is pathogenic. Of considerable interest is the frequently heavy sporulation of the fungus deep within the ovule, where it would naturally be assumed that the oxygen supply is low.—M. P. BACKUS AND G. S. BRYAN (University of Wisconsin).

CONCEPTS AND MISCONCEPTS IN TYMPANIS

The writer has recently read with great interest the paper by Dr. J. W. Groves (Canadian Journal of Botany **30**: 571–631. 1952) on the genus *Tympanis* in which he describes a number of

new species and supplies much added information on other species included by him in the genus. It is a valuable contribution but there is one statement to which the writer wishes to take exception, as might be expected. The statement is as follows: *"Seaver (1951) lists six species of Tympanis but apparently had a misconception of the genus."* Since there are about as many interpretations of the genus as there are workers on the group who is to decide which of these is a misconception? Furthermore as knowledge increases concepts change. Let us point out some of these changes.

The writer began his studies on the Discomycetes more than fifty years ago, his objective being to list in one work the species occurring in North America so far as it is possible for one author to do so. In doing this it was necessary to deal with many genera and one could not help being impressed with the divergence of views regarding them and the changes of view as time went on and knowledge accumulated. We may use as an illustration the old genus *Peziza*. In the early days most all Discomycetes were placed in this genus. As the number increased, species and groups of species were removed and the original genus whittled down until today it includes only a small fraction of the species which it once comprised. Yet at this time it is doubtful if any two workers on this group would have exactly the same concept of the genus *Peziza* itself.

What has happened to *Peziza* has happened to other genera of the Discomycetes. This is especially true of *Tympanis*, the genus in question. In looking over the genera of the Cenangiaceae it will be noted that numerous species formerly placed in the genus *Tympanis* have been removed and relegated to other genera such as *Dermea, Pezicula, Godronia, Cenangella,* and *Dermatella*, largely on the basis of ascospore characters. As a result the genus *Tympanis* has also been reduced until it comprises only a very small percentage of the species originally included. But so far as the writer recalls, those species of Cenangiaceae in which the asci were filled with these minute spore-like bodies and in which the normal ascospores were either absent, undeveloped, or unobserved were retained in the genus and it came to be largely confined to those forms.

According to Groves normal ascospores, or what he terms primary ascospores, have been known in *Tympanis* since the time of Nylander (1868). Apparently they were not generally known for when William Phillips published his British Discomycetes in 1893, twenty-five years later, he listed eight species of *Tympanis*. In the six of these containing the minute spore-like bodies, normal ascospores were unknown or at least not known to him. The other two contained normal ascospores only and one of these had already been removed from the genus by Karsten. What became of the other the writer does not know. The point to be emphasized here is that at that early date the genus *Tympanis* had become restricted almost exclusively to those forms with the so-called secondary spores and in which the normal ascospores were unknown.

This is the concept (or misconcept) early adopted by the writer and hence *Tympanis* had come to be regarded as a form genus in which to place those species of Cenangiaceae in which the normal or primary ascospores were unknown. It was assumed that if and when true ascospores were found the species would be reclassified and placed in the proper genus as indicated by the character of the ascospores. There is a similar situation in the Hypocreales. A number of species in that order produced minute secondary ascospores which often obscured the normal ascospores. For some time these were kept together, but later distributed, according to the character of the ascospores, among the genera *Nectria, Calonectria, Scoleconectria* and *Thyronectria.*

To keep together in the same genus species with the diversity of ascospores reported by Dr. Groves for *Tympanis* would appear to the writer to be inconsistent with the treatment in other groups of Discomycetes. However this is not meant to be in any sense a criticism of the splendid work done by Dr. Groves on this and other genera of the Cenangiaceae but merely an attempt to explain and defend the concept of the genus *Tympanis* as presented by the writer in The North American Cup-fungi (Inoperculates), privately published. The writer claims no originality for this concept, neither does he feel inclined to retract or apologize for it. His views, however, are not binding on anyone else and all are

free to accept or reject as they may see fit.—FRED JAY SEAVER, Winter Park, Florida.

AN UNUSUALLY LARGE FOMES

Mycologists, like fishermen, like to report their largest catches. The giant puffball (*Calvatia maxima*) is certainly a leading contender for honors. Bessey, in his Textbook of Mycology (Blakiston's Son and Co. Phila. 1935), reports a sporophore measuring $1.6 \times 1.35 \times 0.24$ meters. In our local papers photographs of large fruiting bodies of *Polyporus berkeleyi* are to be seen most seasons. Just recently W. H. Wills (Mycologia **45**: 144–145, 1953) has reported such a specimen weighing 45 pounds and measuring up to 26 inches in diameter. A less familiar polypore, *Amauroderma brittonii,* is reported by Vélez (Turtox News **30**: 213, 1952) as measuring 90 cm. in diameter. Even the very abundant and well known *Fomes applantus* is to be listed among the species producing unusually large sporophores. Bessey (Textbook of Mycology) states that spore fruits of this species may attain a diameter of 75 cm. It is the purpose of this note to report an unusually large specimen of this species.

In November, 1942, while on a mycological class field trip, we happened upon a very large bracket of what was tentatively identified as *F. applanatus.* The bracket was attached to a living tree of *Acer rubrum* (red maple) growing in a bog forest near Aurora, Ohio. Using a small ruler the greatest diameter was determined to be 64 cm. This so impressed us that we photographed the specimen along with the 15 cm. rule (FIG. 1). The specimen was not collected, hence the tentative status of the identification. It was our hope that we could locate the bracket the following season and thereby follow its development. World War II interfered. Thanks to its location in a relatively inaccessible bog, no one had destroyed it during my long absence. Nature, however, was not so kind. Upon relocating the spot in July, 1948, we found that the host tree, as well as the perennial sporophore, had died. Decay had so weakened the bracket that it had partially fallen from the tree (FIG. 2). It was in such a condition as to discourage collection or further efforts of identification. We therefore con-

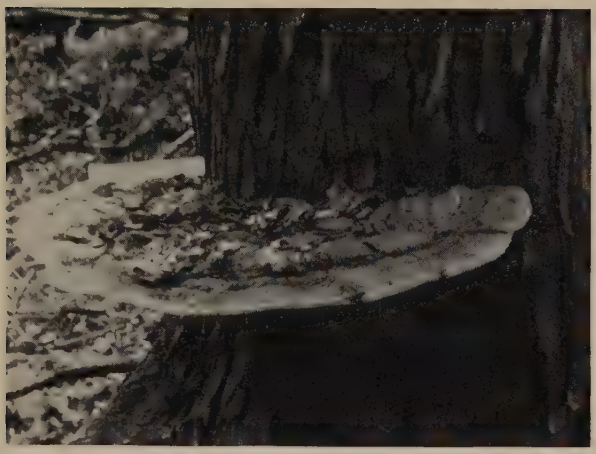

FIG. 1. Bracket of *Fomes applanatus*, with a 15 cm. rule, measuring 64 cm. from side to side. November, 1942. From a kodachrome.

tented ourselves with a new measurement and a new photograph. In order to determine the diameter it was necessary to assemble the fragments and then measure. Measuring the maximum side to side diameter, as in 1942, we found that it had increased from its former 64 cm. to a final measurement of 97 cm. As Vélez

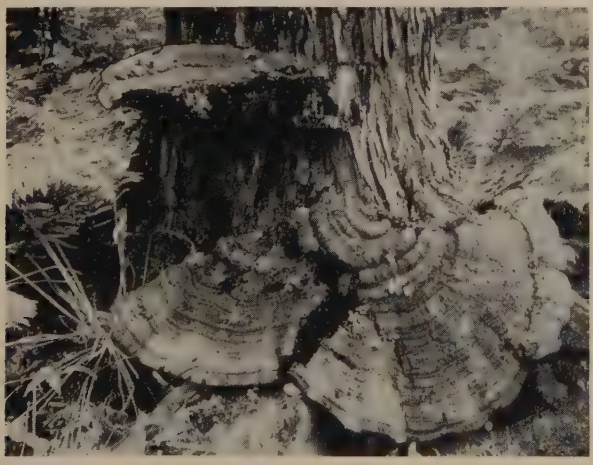

FIG. 2. The same bracket as shown in Fig. 1 as seen in July, 1948, the maximum diameter, before breaking, having been 97 cm. From a kodachrome.

(above) says of his remarkable collection, "It is certainly one of the largest caps known."—J. ARTHUR HERRICK, Kent State University.

EIGHTH INTERNATIONAL CONGRESS OF BOTANY

The VIIIth International Congress will be held in Paris from Friday the 2nd to Wednesday the 14th of July, 1954. There will be a Post-Congress at Nice from July 22nd to the 25th or 26th. A number of excursions will be arranged before, during and after the Congress, including trips to north and equatorial Africa.

All who plan to attend should begin to make their plans. Notices have been sent to all those who may be supposed to be interested and whose names and addresses are available to the secretariat. Any who have not received announcements may secure them by writing to the Secretariat General du 8e Congress International de Botanique, 292, rue Saint-Martin, Paris 3e, France.

Dr. William Chambers Coker, known for his contributions to the knowledge of southeastern trees, in addition to his outstanding work on the fungi, died on June 27, in his eighty-first year.

REVIEWS

PHYTOPATHOLOGIE DES PAYS CHAUDS. Vol. II, by L. Roger (Encyclopedie Mycologique, Vol. XVIII). Pp. 1127–2256, figs. 154–361. Paul Lechevalier, Paris. 1953. Unbound. Price 10,000 francs (about $28.80).

The first volume of this ambitious work was reviewed in MYCOLOGIA **44**: 429. 1952. The second volume, of almost exactly the same size and even more profusely illustrated, covers diseases of tropical plants caused by Ascomycetes and imperfect fungi, arranged in the order of the causal fungi, following a rather conservative and familiar system of classification and with clear and usable keys.

Volume III, which is announced for publication this year, will include bacterial and virus diseases, diseases caused by algae, lichens, and parasitic flowering plants, a general discussion of disease control, arranged by hosts, and the indexes. The last will greatly facilitate the reference use of the work.

Considering the vast scope of the work and its necessarily limited circulation, the price is not excessive for these times, and the amount of information assembled and not readily available elsewhere makes it imperative that all laboratories where plant disease fungi are studied have it available.—G. W. M.

THE FUNGI. A DESCRIPTION OF THEIR MORPHOLOGICAL FEATURES AND EVOLUTIONARY DEVELOPMENT, by Ernst Albert Gäumann, translated by Frederick Lyle Wynd. 420 pp., 440 figs. Hafner Publishing Co., New York. 1952. Price $10.00.

The Gäumann text, published in Switzerland in 1949, needs no introduction to American mycologists. The appearance of an English translation will permit wider circulation and adds another to the group of excellent texts on the fungi now available in English.

The translator has followed the German text very closely, but

has rendered it into clear, idiomatic English. The most striking changes are in the bibliography, where the titles are given in full, and in the diagrams of life-cycles, which have been redrawn in a form clearer, to American readers at least, than in the original.

There are a few errors or ambiguities in translation, including those of the legends for Figs. 52, 203 and 242. The statement that "*Ascocorticium* produces a rind" (p. 229); that the endoperidium of *Astraeus* "lies free from the gleba" (p. 300) and the reference to the maturation of the gleba of *Hysterangium* (p. 306) are examples of faulty translation. The frequent reference to developmental trends within a group as evolutionary tendencies can be defended but perhaps over-emphasizes what is meant in the German original. These are relatively minor matters. It is well to have Gäumann's important book available to those to whom the original language is a barrier; for critical reference the original is readily available.—G. W. M.

PLANT DISEASES, by F. T. Brooks. Second edition. xii + 457 p., 62 figs. Oxford University Press, London, New York, Toronto. 1953. Price $7.50.

As indicated, this volume is a revision of a book published by Dr. Brooks in 1928. In general the arrangement is like that of the first edition: a brief introduction covering the general aspects as to cause of plant disease, their symptoms and their dissemination, their epidemic and sporadic occurrence, the host parasite relationships and environment, and ending with control and legislation on plant disease. Following this are chapters on nonparasitic and virus and bacterial diseases. Diseases caused by Actinomycetes and Plasmodiophorales precede the bulk of the discussion, which is given over to diseases caused by fungi arranged in mycological sequence. The book closes with a chapter on diseases caused by algae and one on fungicides. Excellently selected lists of references accompany each chapter.

The author has packed an immense amount of information into the available space and has shown good judgment in his selection of the material to be added to his earlier treatment.—JOSEPH C. GILMAN.

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MICROASSAY OF ANTIFUNGAL PROPERTIES OF STEROID HORMONES AND OTHER COMPOUNDS[1]

J. E. TARBET, M. OURA, AND THOMAS H. STERNBERG[2]

(WITH 9 FIGURES)

Oster and Golden (25) have stated that assays of fungicides modeled after the "germination tests" employed by plant pathologists would meet some of the needs of pharmacology provided suitable indicator fungi are found among the pathogens of mammals. Species of *Candida* possess certain obvious advantages as indicators of this class. Large numbers of viable spores are regularly obtainable in homogeneous suspension from cultures of *Candida,* and risks of accidental laboratory infection are not great with this organism.

The principal purpose of this study has thus been to apply the enumeration in cultures of the budding cells of a pathogenic strain of *Candida tropicalis* as a measure of the growth of the organism in the presence of various compounds. The effects of a number of steroid hormones and allied substances, as well as of active nonsteroid compounds, on the growth of *C. tropicalis, Microsporum fulvum, Scopulariopsis brevicaulis* and *Saccharomyces cerevisiae*

[1] From the Medical Service, Veterans' Administration Hospital, and the Division of Dermatology, Department of Medicine, University of California School of Medicine at Los Angeles.

[2] This research was supported in part by a grant-in-aid from Ciba Pharmaceutical Products, Inc., Summit, New Jersey. The authors wish to acknowledge their indebtedness for technical assistance to Mr. Frank Pero.

[MYCOLOGIA for July–August (45: 481–626) was issued August 24, 1953]

have been examined. Included are (a) a description and an evaluation of the technic employed, (b) a comparison of the median effective dose values (M)[3] obtained with some of the compounds, and (c) a preliminary investigation of the effects of combinations of active drugs on the growth of *C. tropicalis*.

More information concerning the effect of steroid compounds on the growth of fungi appears to be desirable. A number of investigators (4, 17, 24, 28) have noted enhancement of fungus infections in experimental animals when the steroid hormone, cortisone, was administered. Opinion in general appears to be that cortisone and adrenocorticotropic hormone (ACTH) may exert an unfavorable effect in any class of deep infections (1, 2, 3, 9, 11, 18, 33, 34, 35, 38, 39). It has not been suggested that the disease microorganisms are in any way directly affected by these hormones, but that the resistance of the host is impaired.

The influence of the androgens and estrogens in infectious diseases may be of a different order than that of cortisone and ACTH. Frazier *et al.* (8), Hudson and associates (16), and von Haam and Rosenfeld (12) are among those who have noted improvement in systemic bacterial or viral infections when sex hormones were given. Benefits in superficial fungus infections have been noted, sometimes as a side effect of the parenteral administration of the sex hormones for other conditions (10, 30, 32). The benefits have been ascribed to such changes in the skin, hair and nails as normally occur at puberty, but which may also follow treatment with androgens or estrogens.

Some steroid hormones can affect the growth of fungi directly. Reiss (29) was able to induce trichophytosis *rubrum* in the castrated rabbit, and to limit the disease by topical application of sex hormones (30). He also demonstrated the *in vitro* fungistasis of *Trichophyton rubrum* (*purpureum*) and *Trichophyton mentagrophytes* by testosterone, estradiol and desoxycorticosterone (31). Hruszek (15) had shown earlier that anterior pituitary and ovarian follicular preparations will arrest the cultural development of *T. mentagrophytes* and *Microsporum audouini*.

[3] M is defined as the concentration of drug which reduces the number of growing organisms to one-half.

It is also of interest that the inclusion in the culture medium of stilbestrols and other synthetics with physiological activities like those of the sex hormones, has been found to modify the growth of fungi and bacteria (5, 13, 31). Stilbestrol has also been used successfully to protect mice in streptococcal infections (6).

Mammalian sex hormones may affect some fungi in the actual capacity of hormones. Plumb and Durrell (26) reported that traces of estrin in the culture medium suppressed the production of gametangia and zygospores in *Rhizopus nigricans* without interfering with vegetative growth.

METHODS AND MATERIALS

Indicator Organisms. A number of "monilia" isolates from different sources were examined in a preliminary survey before choice was settled on an organism obtained from an axillary abscess and identified as *Candida tropicalis* (Castellani) Berkhout.[4] This microorganism was selected for study because of its rapid growth in the medium employed, and its large cell size. *Microsporum fulvum* Sabouraud, *Saccharomyces cerevisiae* Hansen and *Scopulariopsis brevicaulis* (Saccardo) Bainier were also used as indicators in some of the experiments. Stock cultures of the organisms were maintained by a serial transfer on Sabouraud's agar slants in screw-cap culture bottles of the type employed by Wilson and Plunkett (40).

Assay Medium. The following broth was employed in all of the experiments:

Dextrose	20 gm
Glycine	2 gm
$MgCl_2 \cdot H_2O$	2 gm
Phosphate buffer [5] pH 6.4	200 ml
Distilled water	800 ml

[4] Intraperitoneal inoculation of mice with this fungus, using eight million cells, resulted after four weeks in spleen, liver and kidney lesions. Application of the procedures for the speciation of *Candida* proposed by Martin *et al.* (19) and Martin and Jones (20) placed it in the *Candida tropicalis* group producing large gray colonies with a mycelial fringe on blood agar at 37° C, acid and gas in glucose, sucrose and maltose but not in lactose, and developing no chlamydospores on cornmeal agar.

[5] Sorensen phosphate standards. *Practical Physiological Chemistry.* P. B. Hawk and O. Bergeim, 11th Ed., 1944.

Preparation of the Test Compounds.[6] The compounds used in the experiments were weighed analytically. Volumetric technic was employed to obtain the dilutions of the substances required, using the assay medium as diluent. Five-tenths ml volumes of each dilution of each substance, at double the final concentrations desired, were pipetted into as many replicate tubes as required to assemble the experiments. To the tubes was then added 0.5 ml of medium containing known numbers of fungus cells.

All of the test substances were soluble in the medium at the low concentrations (up to about ten parts per million (ppm)). Those substances which were not soluble in the medium at the higher concentrations were first dissolved in a minimum of acetone, the required volume of medium was then added, and the proper volume recovered by evaporation. In most instances this treatment resulted in a clear solution. When it did not (α-estradiol, progesterone, testosterone, testosterone undecylenate), a fine suspension was obtained.

Sterile glassware, instruments and medium were used in the preparation of the test compounds and in assembling the experiments, but subjecting the compounds themselves to the ordinary processes of sterilization was avoided because some of them were

[6] The following crystalline substances were furnished to the authors by Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

Cholesterol
Desoxycorticosterone
Desoxycorticosterone glucoside
Diethyl-2-hydroxyethyl-(3-keto-Δ^4-17-pregnadienyl) NH_4BR
α-estradiol
Pregnenolone
Progesterone
17-amino-Δ^4-androstene-3-one HCl
17-amino-Δ^4-androstene-3-ol
Testosterone
Testosterone undecylenate
3-keto-21-(piperidyl)-Δ^4-17-pregnadiene HBr
20-amino-3β-hydroxy-5-pregnene HCl
o-dichloracetyloxine
3,4-dichloro-ω-trichloromethyl acrylophenone
Bradosol(R) (phenoxyethyl-dimethyl-dodecyl NH_4Br)

Cortisone acetate and hydrocortisone acetate were obtained from Merck and Company, Inc., Rahway, New Jersey.

heat labile. This did not result in contamination, possibly owing to the fact that all of the test compounds were stored in sealed vials for several weeks before use.

Preparation of Inocula. Inocula for the tests were prepared by flooding the surface of 15-day-old stock cultures of the fungi with 6 ml of medium. Pure suspensions of the conidia of *M. fulvum* and *S. brevicaulis* were obtained in this way. The cultures of the yeast-like fungi yielded 80–95% unicellular forms. Five ml of the spore suspensions were transferred to 50 ml flasks containing 25 ml of medium. After thorough shaking, the fungus cells were counted, using the leukocyte chamber of the hemacytometer for the yeast-like cells and a drop of inoculum beneath a coverslip (22 mm^2, No. 1 thin) for enumerating the conidia of the two mycelial forms. Suitable dilutions of the fungus cell suspensions were then prepared to contain the following numbers of individuals:

Organism	*Numbers of cells in inoculum*
C. tropicalis	200–400 per cmm
M. fulvum	6–10 per field (× 100)
S. cerevisiae	200–400 per cmm
S. brevicaulis	6–10 per field (× 430)

The numbers of cells in the assay cultures at the start of incubation were, of course, half those indicated above, because the inocula prepared as above were further diluted in the test tubes with medium containing suitable concentrations of the test compounds. These numbers of cells gave rise to cultures of a density convenient for enumeration after convenient intervals of incubation (6–7 hours for *S. brevicaulis;* 13–16 hours for *C. tropicalis;* 17–24 hours for *M. fulvum* and *S. cerevisiae*).

Growth and germination curves for the microorganisms were obtained in a preliminary experiment in order to ascertain the most satisfactory intervals of incubation. Many replicate cultures of each organism were prepared in one ml volumes of the assay medium in serologic test tubes. Incubation was carried out at 28–30° C with constant shaking (Kahn type shaker; 275–285 oscillations per minute through 1½″ stroke) as in all subsequent experiments. The population per unit volume of the yeast-like

fungi was found at intervals of hours, using the leukocyte chamber of the hemacytometer, until the values obtained approached the asymptote. With *M. fulvum* and *S. brevicaulis* 200 conidia were examined at intervals and the numbers of germinating cells recorded.

Counts of the total population in aliquots from replicate cultures of the yeast-like fungi varied more than counts of the blastosporulating fraction of the population only. It is believed that this failure of counts of total population to "check" resulted from premature mechanical separation of some of the blastospores due to the shaking. As the blastospores of *C. tropicalis* are multiple in number, the premature separation of some of them does not prevent the recognition of actively growing cells. Thus, with this organism the number of blastosporulating fungi and not the total number of cells, was selected as the more satisfactory measure of growth. To arrive at an estimate of the variation to be expected in such cell counts of yeast-like fungi, the mean number of blastospores per unit volume was found in 25 groups of three cultures of *C. tropicalis* which had been prepared as replicates. The coefficient of variation was 6.0%.

Assay Technic. In obtaining dosage-response data and estimating M values, the number of germinating individuals in 200 was found for *M. fulvum* and *S. brevicaulis*, and the number of budding individuals of *Candida* or *Saccharomyces* per cubic millimeter, for several levels of each drug studied, using the incubation intervals and counting procedures described above. Triplicate cultures of each microorganism at each level of the test compounds provided satisfactory values.

As dozens of culture tubes were incubated simultaneously in each experimental "run," it was necessary to stabilize their contents on removal from the incubator during the interval required to count the spores in all of them. This was accomplished by refrigerating the cultures at 4° C. It could also be accomplished by adding a measured drop of formaldehyde to each of the culture tubes.

EVALUATION OF TECHNIC

Several experiments were undertaken for the purpose of evaluating the technic.

(a) To estimate the extent to which the M values for a known antifungal compound are reproducible, four dose-response curves were obtained for Bradosol(R) with *C. tropicalis,* using different inoculum densities, and solutions of the drug separately prepared. Incubation was carried out for different lengths of time from 14 to 18 hours. The range of M values obtained was 0.8 ppm, with a mean for the four experiments of 2.2 ppm.

(b) A comparison of the sensitivity of the counting technic with that of photoelectric turbidimetry in the detection of early differences in the growth response of *C. tropicalis* was made. The Coleman spectrophotometer was utilized, employing a wavelength of 400 millimicra. The results of this comparison are tabulated below.

Growth of *C. tropicalis*

Measured by Photoelectric Turbidimetry and by Direct Cell Counts

Incubation time (hours)	*% Transmission*	*Blastospores per cubic millimeter*
0	99.8	32
4	100.0	60
6	100.0	115
8	100.0	190
11	99.6	458

Differences in growth were discernible by the counting technic seven hours earlier than by turbidimetry.

(c) It was also necessary to test whether indications of activity for certain drugs, obtained by direct cell counting, were verifiable by other methods of estimating growth response, and whether drugs active for *C. tropicalis* were also active for other kinds of fungi under the same and other conditions.

(1) The M value for the steroid compound 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide was estimated with *C. tropicalis* to be in the vicinity of 5 ppm. Cortisone acetate and pregnenolone, on the other hand, were inactive at this level, but at the levels of about 10 ppm and above, they accelerated the growth of this fungus. These three steroid compounds and two other active compounds, undecylenic acid and 3,4-dichloro-ω-tri-

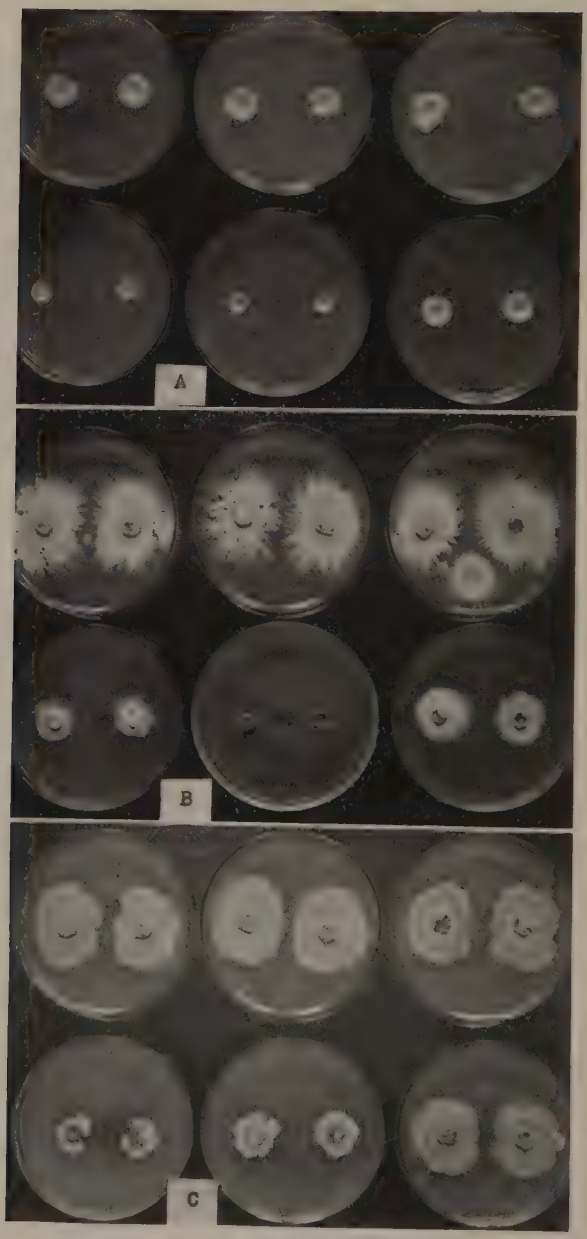

FIG. 1. Growth on Sabouraud's agar plates as a measure of drug activity. (A) *C. tropicalis*, (B) *M. fulvum*, (C) *S. brevicaulis*. From left to right the plates contained 100 ppm of each of the following: no drug, cortisone acetate, pregnenolone, 3,4-dichloro-ω-trichloromethyl acrylophenone, 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide, undecylenic acid.

chloromethyl acrylophenone, were incorporated separately in agar plates. The growth of *C. tropicalis, M. fulvum* and *S. brevicaulis* on these plates in the presence of 100 ppm of each compound is shown in FIG. 1. The general response of the microorganisms to the drugs was the same as by the counting technic with *C. tropicalis*. Growth on the agar plates appeared to be a less sensitive gauge of direction and extent of drug activity. Enhancement of the plate cultures by cortisone and pregnenolone could just be discerned.

(2) Two of the steroid compounds which exhibited activity as measured by cell counts with *C. tropicalis* and three commercial antimicrobial agents, were tried against *M. fulvum, S. cerevisiae* and *S. brevicaulis* as well, at levels 1 and 100 ppm. The values are given in blastospores per cubic millimeter for *C. tropicalis* and *S. cerevisiae*, and in germinating conidia per 200 for *M. fulvum* and *S. brevicaulis* in the table.

EFFECTS OF ANTIFUNGAL SUBSTANCES ON THE GROWTH OF FOUR FUNGI

Substance	*C. tropicalis*			*M. fulvum*			*S. brevicaulis*			*S. cerevisiae*		
	Parts per million of substances in cultures											
	0.0	1.0	100.0	0.0	1.0	100.0	0.0	1.0	100.0	0.0	1.0	100.0
Orthocide(R)	778	635	12	34	6	0	116	146	125	465	155	115
Triton A(R)	535	492	471	50	71	40	146	143	146	228	132	105
Vioform(R)	535	544	16	50	45	0	146	156	0	228	28	15
17-amino-Δ^4-androstene-3-one HCl	535	720	58	50	29	10	146	146	125	228	130	107
20-amino-3β-hydroxy-5-pregnene HCl	535	329	39	50	53	9	146	156	72	228	140	36

The pattern of response was similar in all four organisms; the non-pathogens were somewhat less sensitive as indicators.

EXPERIMENTAL RESULTS

Screening of Test Compounds. Measurement of the growth of the test organisms in the presence of 1 and 100 ppm of the various steroids and of the non-steroid compounds which were included for comparison, resulted in the values listed in TABLE I.

TABLE I

GROWTH OF CANDIDA TROPICALIS AND MICROSPORUM FULVUM WITH STEROID AND OTHER COMPOUNDS

Compounds (Grouped according to activity)	*C. tropicalis**			*M. fulvum***		
	Parts per million of compound in culture medium					
	0.0	1.0	100.0	0.0	1.0	100.0
(*a*) *Stimulation*						
Cortisone acetate	1192	1235	2215	50	96	100
Hydrocortisone acetate	710	1122	1030	72	72	76
Testosterone	1192	1320	1578	72	81	100
Testosterone-diethyl-aminoethyl carbonate	1075	1209	2198	72	75	80
(*b*) *Equivocal*						
Cholesterol	535	540	476	50	78	96
Pregnenolone	1192	1216	1772	72	72	69
17-amino-Δ^5-androstene-3-ol	1192	1170	1568	72	65	46
Testosterone undecylenate	1075	1166	1568	72	68	58
(*c*) *Slight Inhibition*						
α-estradiol	1192	1186	1082	72	70	68
Desoxycorticosterone gluccside	1075	951	562	72	72	66
Diethyl-2-hydroxyethyl-(3-keto-Δ^4-17-pregnadienyl) NH_4Br	1075	1232	518	72	71	42
Progesterone	1075	1047	998	76	70	64
(*d*) *Moderate Inhibition*						
Desxoycorticosterone	665	695	365	72	64	0
17-amino-Δ^4-androstene-3-one HCl	1070	1059	116	50	34	10
3-keto-21-(piperidyl)-Δ^4-17-pregnadiene HBr	1192	1140	188	72	55	48
20-amino-3β-hydroxy-5-pregnene HCl	1070	658	78	50	23	9
(*e*) *Non-steroid Compounds*						
Bradosol(R)	945	427	0	50	20	0
o-dichloroacetyloxine	1015	706	0	50	31	0
3,4-dichloro-ω-trichloromethyl acrylophenone	1015	698	0	50	29	0
Undecylenic acid	910	1170	610	52	48	0

* Values represent numbers of blastospores per cubic millimeter of culture.

** Values represent numbers of germinating macroconidia in two hundred.

Enhancement of fungus growth by antimetabolites frequently occurs at levels lower than those which are toxic (**41**). It may be this effect which is seen with the compounds of group (a) in TABLE I. The effect emerges more clearly with the steroids of group (b). The critical level for inhibition of the indicator organisms by the compounds of group (b) evidently lies in the re-

gion of 100 ppm. Acceleration of the growth of one of the indicators and inhibition of the other may be noted with these compounds. A number of steroids (Groups(c) and (d)) possessed some antifungal activity.

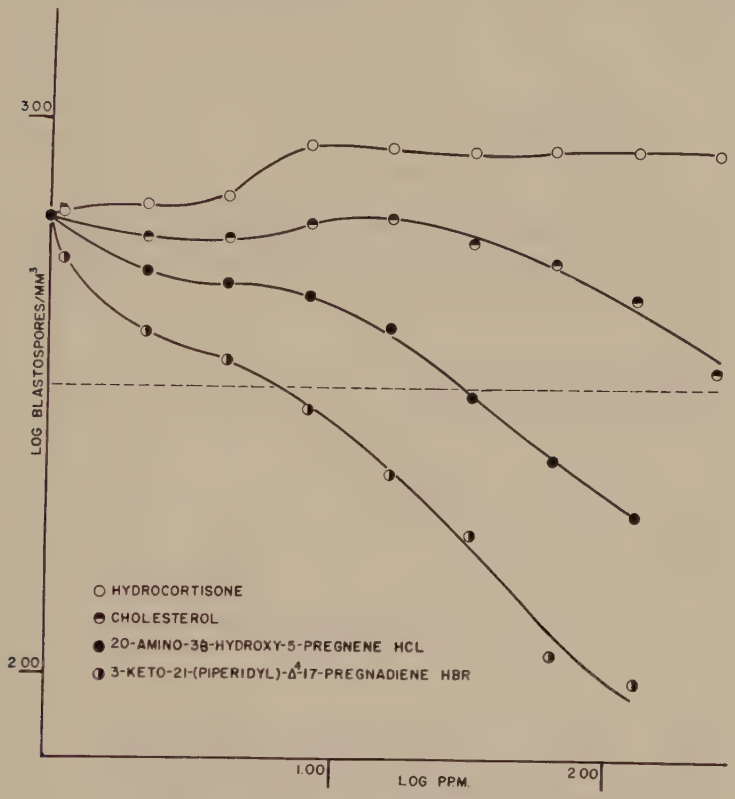

FIG. 2. *C. tropicalis.* Dosage-response curves for some active steroids. The broken line intersects the curves at the M values.

Comparison of M Values. Using *C. tropicalis* as indicator, dosage-response data were obtained in more detail for cholesterol, hydrocortisone acetate, testosterone-diethyl-aminoethyl carbonate, the steroid compounds of group (d) and the non-steroids of group (e) (TABLE I). The acceleration of growth in the presence of hydrocortisone acetate as indicated in TABLE I was confirmed (FIG. 2). A similar acceleration was seen with testosterone-diethyl-aminoethyl carbonate. Median effective dose estimates

for desoxycorticosterone, 17-amino-Δ^4-androstene-3-one monohydrochloride and cholesterol were found to be in the vicinity of or greater than 100 ppm. Only five compounds of the entire group possessed any notable activity against *C. tropicalis*. The M values obtained for these five substances were as follows:

Compound	*Median effective dose ppm*
Bradosol(R)	2
o-dichloracetyl oxine	1
3,4-dichloro-ω-trichloromethyl acrylophenone	3
3-keto-21-(piperidyl)-Δ^4-17-pregnadiene HBr	5
20-amino-3β-hydroxy-pregnene HCl	25

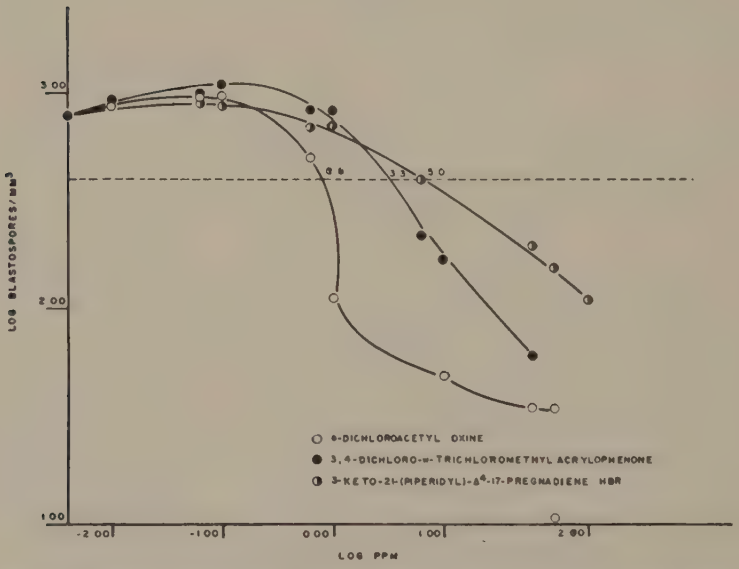

FIG. 3. Dosage response of *C. tropicalis* to three antifungal compounds. The broken line intersects the curves at the M values.

Effects of Mixtures of Antifungal Substances on the Growth of C. tropicalis. (a) Dosage-response data were obtained for o-dichloroacetyl oxine, 3,4-dichloro-ω-trichloromethyl acrylophenone, and the steroid, 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide, employing three different media. The media were the assay broth, and two portions of the same broth to which were

added sufficient Bradosol(R) to give concentrations of 0.1 and 10.0 ppm, respectively.

A comparison of the response curves in FIGS. 3 and 4 discloses that in the presence of a trace of Bradosol(R) the M values obtained for the other three compounds were somewhat lower than in basal medium only. Traces of this fungicide in the medium enhanced the activity of the other three drugs to a moderate degree.

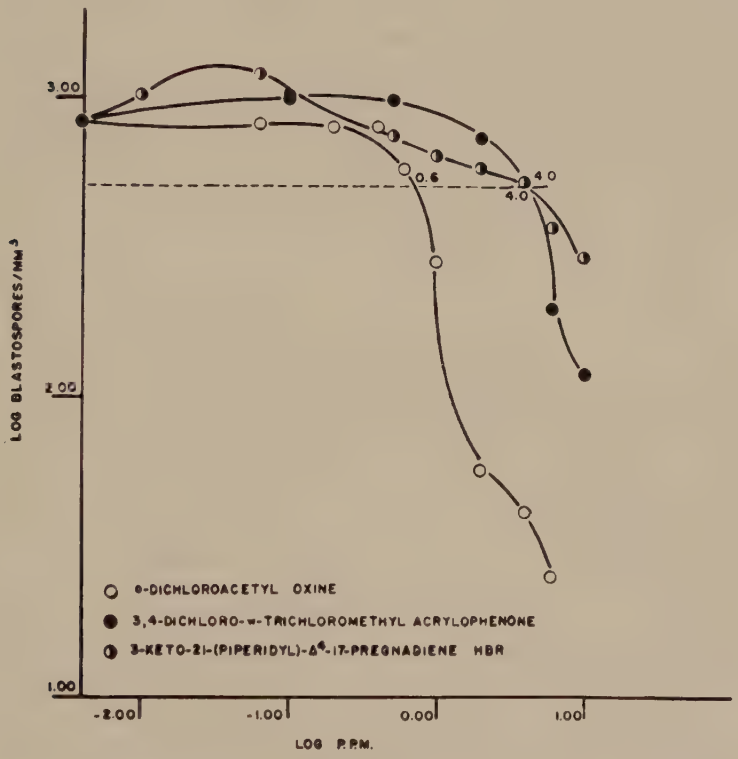

FIG. 4. Dosage-response of *C. tropicalis* to three antifungal compounds in medium containing 0.1 ppm Bradosol(R). The broken line intersects the curves at the M values.

In the presence of 10 ppm (approximately 5 M) of Bradosol(R), the M values for each of the other three drugs were considerably lower (FIG. 5) than in the basal medium. In addition, a curious effect was seen. There was now a choice of M values as follows:

Compound	Median effective dose (ppm) Lower dose	Higher dose
o-dichloroacetyl oxine	0.02	1.0
3,4-dichloro-ω-trichloromethyl acrylophenone	0.05	2.0
3-keto-21-(piperidyl)-Δ^4-17-pregnadiene HBr ..	0.09	1.0

The advantages seemingly offered by the low median effective doses for the three drugs in this experiment are illusory. Approximately five M of the Bradosol(R) compound were present in the assay broth. Mixture of any of the other three drugs in amounts up to one or two parts per million with 10 ppm of Bradosol(R) interfered with the fungicidal action of that compound.

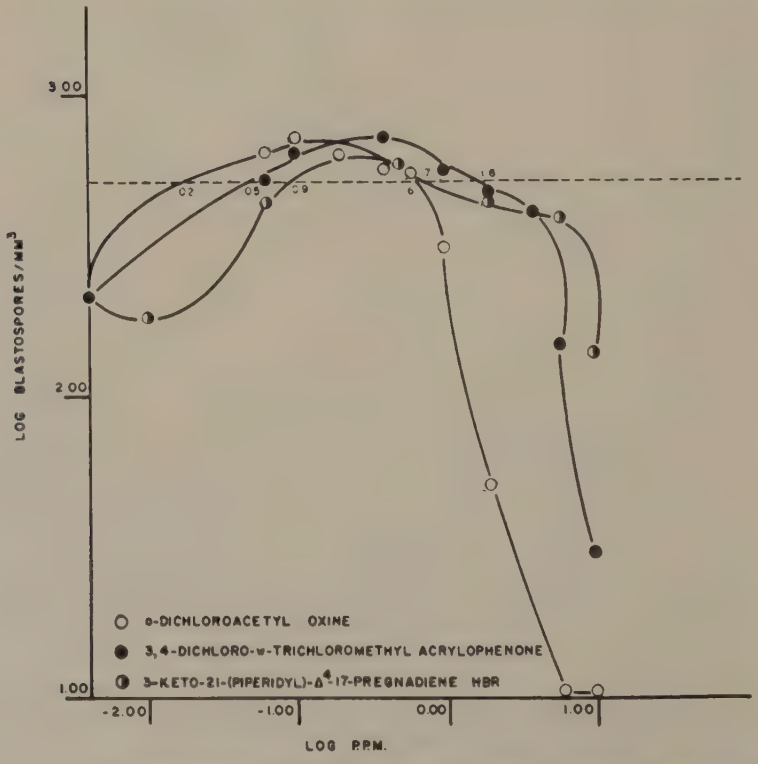

FIG. 5. Dosage-response of *C. tropicalis* to three antifungal compounds in medium containing 10 ppm Bradosol(R). The broken line intersects the curves at the M values.

(b) Dosage-response data for *C. tropicalis* with 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide were obtained in basal medium and in media containing, respectively, 0.1 and 10.0 ppm of 20-amino-3β-hydroxy-5-pregnene monohydrochloride (FIG. 6).

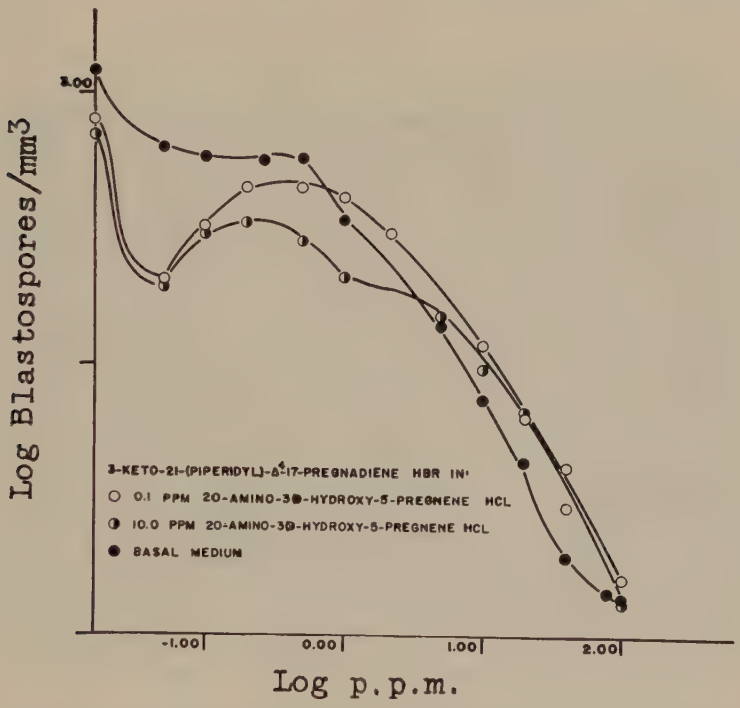

FIG. 6. *C. tropicalis.* Dosage-response to 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide in basal medium and media containing 20-amino-3β-hydroxy-5-pregnene monohydrochloride.

Nothing appeared to be gained by admixture of 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide in amounts exceeding one or two parts per million with either 0.1 or 10.0 ppm of the other steroid. But mixture of fractional parts per million with either 0.1 or 10.0 ppm of 20-amino-3β-hydroxy-5-pregnene monohydrochloride resulted in antifungal activity greater than the activities of either of the two compounds alone at any of these levels.

(c) Dosage-response data for *C. tropicalis* with 20-amino-3β-hydroxy-5-pregnene monohydrochloride were similarly obtained, employing basal medium and media containing, respectively, 0.1 and 10.0 ppm of the other steroid (FIG. 7). In this experiment, the most desirable synergistic effect was seen with the combination,

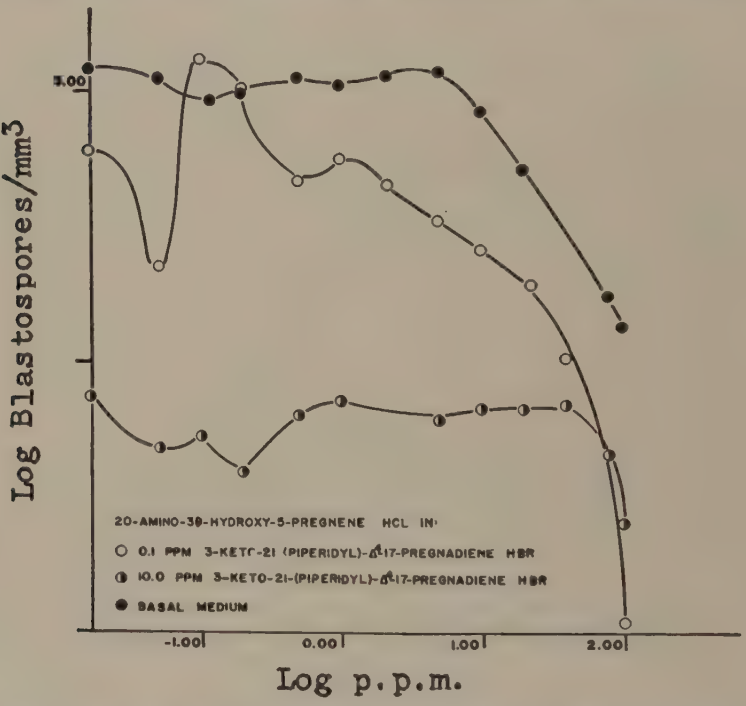

FIG. 7. *C. tropicalis*. Dosage-response to 20-amino-3β-hydroxy-5-pregnene monohydrochloride in basal medium and media containing 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide.

0.05 ppm of the first pregnene derivative with 0.1 ppm of the second. The antifungal activity of 20-amino-3β-hydroxy-5-pregnene monohydrochloride was enhanced to a considerable extent at almost all levels by the inclusion of 0.1 ppm of 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide in the medium.

In medium containing 10 ppm (approximately 2 M) of the second compound, on the other hand, little or nothing was gained by the addition of 20-amino-3β-hydroxy-5-pregnene monohydrochloride.

(d) To observe whether some of the drug combinations found to act in synergism or antagonism with *C. tropicalis* would affect the growth of another fungus similarly, Sabouraud's agar plates containing some of the combinations were prepared, and the growth on them of *M. fulvum* was observed (FIGS. 8, 9). These plates were inoculated with discs cut with a No. 3 cock-borer from 3-weeks-old colonies of *M. fulvum* on Sabouraud's agar.

FIG. 8 (above). Drug synergism with *M. fulvum*. Left to right: control; 0.05 ppm 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide: 10 ppm 21-amino-3β-hydroxy-5-pregnene monohydrochloride; (1) plus (2).
FIG. 9. (below) Drug antagonism with *M. fulvum*. Left to right: control; 2 ppm Bradosol$^{(R)}$; 5 ppm 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide; (1) plus (2).

The dermatophyte evidenced particular sensitivity to 20-amino-3β-hydroxy-5-pregnene monohydrochloride. A quantity less than half the M value for *C. tropicalis* inhibited radial growth considerably. Combination of 10.0 ppm of this steroid with 0.05 ppm of 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide (a trace which by itself actually accelerated the growth of the fungus) affected the development of the organism more than either drug

alone (FIG. 8). With combinations of 2.0 ppm of Bradosol(R) with 5.0 ppm of 3-keto-21-(piperidyl)-Δ^4-17-pragnadiene monohydrobromide, another effect was seen. The separate antifungal activities of the drugs were not additive. The combination produced an intermediate degree of inhibition (FIG. 9).

DISCUSSION

The aim of the laboratory assay of the fungicidal activity of chemical compounds is to distinguish and measure differences in potency and to reduce these differences to figures of performance which can be conveniently compared. Our trial of *Candida tropicalis* as a test organism has shown this pathogen to be sensitive and sufficiently manageable as an indicator to possess real value as a tool in the estimation and comparison of the potencies of a variety of compounds and mixtures.

Several steroids which were assayed in these experiments were found to stimulate the growth of the fungi at relatively low concentrations, while interfering with their development at higher levels. Wooley (41), Foster (7) and others have put forward explanations of this capacity of many antimetabolites to stimulate growth at sub-toxic levels. Some of the steroids, including cortisone and hydrocortisone, consistently accelerated the growth of the cultures in the concentrations employed. Probably this increase of growth bears no relation to the enhancement of fungus infections which has occasionally been noted in connection with cortisone therapy.

It would not be rewarding to undertake a full discussion of the many mechanisms whch have been proposed to explain the occasional enhancement of infection with cortisone. It should be pointed out, however, that delay of the inflammatory reaction (21, 22, 23), interference with the normal processes of healing (27), and interference in general with the function of the reticuloendothelial and lymphatic systems (34, 39), have been among the explanations offered. Most authorities agree that cortisone acts in some way to increase host susceptibility to infectious microorganisms, but has no direct influence on the agents of disease *in vivo*.

If the dosage-response curves obtained for all of the steroids were arrayed as in FIG. 2, it was seen that cholesterol itself inhibited the test organisms to some extent, and that the nitrogen-containing steroids possessed the greatest activity. Further correlation to the differing activities with the different structures of the steroids would be difficult. Probably the cyclopentanophenanthrene nucleus itself contributes to the antifungal properties of some of these compounds. It is known that few fungi are able to oxidize the steroid molecule (**36, 37**), and that some of the many steroid substances synthesized by fungi themselves represent waste products (**7**) capable of limiting the growth of the organisms which produce them. It is thus not surprising that some mammalian steroid hormones and related substances, analogous in structure to natural mycosterols also affect the growth of fungi.

Only a few of the steroids possessed any considerable antifungal potency. These were desoxycorticosterone, 17-amino-Δ^4-androstene-3-one-monohydrochloride, 20 amino-3β-hydroxy-5-pregnene monohydrochloride and 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide. The slopes of the dosage response curves obtained with these compounds were gradual, unlike the steep "drops" seen with protoplasmic poisons and other highly toxic fungicides. Such slopes have been associated with antifungal agents possessing the differential toxicity for host organism and fungus parasite which characterizes many useful antibiotics. Further study of antifungal steroids, including animal experimentation, is, of course, essential before conclusions concerning their practical usefulness can be drawn. It is of interest that Kull *et al.* (**42**) have also recently found that these and other amino steroids possess antimicrobial activity.

Some synergistic effects of interest were seen when, instead of single compounds, mixtures of some of the drugs were assayed. A sub-toxic trace of Bradosol(R) induced lower M values when combined with certain other drugs. Admixture of Bradosol(R) with other drugs in a quantity exceeding its own M value resulted, on the other hand, in reduced antifungal activity. This appears to be an instance illustrating the common observation that synergism may occur at some relative proportions and antagonism

at other proportions with the same compounds. There was also synergistic inhibition of the indicator microorganisms by mixtures of 3-keto-21-(piperidyl)-Δ^4-17-pregnadiene monohydrobromide and 20 amino-3β-hydroxy-5-pregnene monohydrochloride (FIGS. 6, 7). Of particular interest were the antifungal effects of mixtures containing traces of these steroids so slight as to be inactive separately. It seems possible that they operate as mutual activators.

SUMMARY

The antifungal activities of a number of steroids and other compounds were compared by a spore-counting method, employing *Candida tropicalis* as the principal test organism. This organism proved to be of particular value as an indicator in these tests.

While most of the steroid compounds possessed some activity, only a few exhibited any notable property as fungicides. With the exception of desoxycorticosterone, the active steroids were nitrogen-containing derivatives of sex hormones.

A few steroid hormones, including cortisone, accelerated the growth of the fungi. Probably this acceleration is not related to the enhancement of fungus infections which has sometimes been noted in connection with cortisone therapy for the relief of other conditions.

Certain mixtures of the steroids and other compounds inhibited the growth of the fungi synergistically. In other mixtures, the antifungal potencies of the components were antagonistic.

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DIFFERENTIATION OF SPECIES OF TAPHRINA IN CULTURE. UTILIZATION OF NITROGEN COMPOUNDS

A. J. Mix

A number of species and of host-forms of *Taphrina* have been brought into pure culture over a period of several years. Since various species and various isolates show only minor differences in colony-characters, and many of them are much alike in morphology of cells formed in culture, other methods of differentiation need to be sought.

In their characterization of genera and species of yeasts, Stelling-Dekker (5), Lodder (1) and other workers have included the ability of the fungi to utilize certain compounds of nitrogen. Species of *Taphrina* in culture behave as yeasts. An unknown species of *Taphrina* in culture would in fact have to be identified as a species of *Torulopsis* as defined by Lodder (l.c.) or by Lodder and Kreger-van Rij (2). Nothing yet published gives any suggestion as to a means of differentiating between the genera *Taphrina* and *Torulopsis* as they appear in culture.

The purpose of the work reported here was to learn whether utilization of various compounds of nitrogen could be used as a basis for differentiation between species of *Taphrina* and between host-forms within one species. It was also hoped to discover whether any significant differences existed between species of *Taphrina* and certain species of non-sporulating yeasts.[1]

[1] A preliminary report of this experimentation was read at the Seventh International Botanical Congress at Stockholm in 1950 and a summary of results is being published in the Proceedings of the Congress. Since 1950, with repetition of a number of experiments some compounds believed to be unavailable to certain fungi have been found usable. The summary mentioned above is therefore incorrect in certain details, but statements made regarding agreement or disagreement in behavior between different species and different host forms do not require correction.

The choice of these yeasts was prompted by the comparative studies made by Roberts (4) with *Taphrina deformans* and *Torulopsis pulcherrima*. The intent was to include representative species of the genus *Torulopsis*. However *Torulopsis pulcherrima* was transferred by Lodder (l.c.) to the genus *Candida*. Of the twelve other fungi chosen six have been recently assigned by Lodder and Kreger van-Rij (2) to the genus *Cryptococcus*, and two to *Candida*. The remaining four are species of *Torulopsis* though the culture of *T. rotundata* used, since it was obtained from Centraalbureau voor Schimmelcultures, may have been the culture studied by Lodder and Kreger-van Rij (l.c.) and found by them to be a strain of *Cryptococcus diffluens* (Zach) Lodder and Kreger-van Rij.

Cultures used: In the following list of cultures those designated by number were used in all the experiments. Additional isolates, usually made at the same time, were used in a number of cases where experiments were repeated. These additional isolates are designated by letter as a, b, etc. in parentheses following the culture number. Original isolations were by the author, unless otherwise designated.

Culture No.
1. (a) *T. americana* Mix. *Betula fontinalis* Sarg.
2. *T. betulina* Rostr. *Betula intermedia* Thomas.
3. (a) *T. betulina*. *B. intermedia*. Formerly *T. Lapponica* Juel.
4. (a) *T. betulina*. *B. pendula* Roth. Formerly *T. turgida* (Sadeb.) Giesenhag.
5. *T. caerulescens* (Mont. and Desm.) Tul. *Quercus alba* L.
6. *T. caerulescens*. *Q. bushii* Sarg.
7. *T. caerulescens*. *Q. coccinea* Muench.
8. *T. caerulescens*. *Q. ilicifolia* Wangh.
9. *T. caerulescens*. *Q. kelloggii* Newb. Isolated by H. N. Hansen.
10. *T. caerulescens*. *Q. laurifolia* Michx.
11. *T. caerulescens*. *Q. macrocarpa* Michx.
12. *T. caerulescens*. *Q. marilandica* Muench.
13. *T. caerulescens*. *Q. maxima* Ashe.
14. *T. caerulescens*. *Q. maxima*.
15. *T. caerulescens*. *Q. nigra*. L.
16. *T. caerulescens*. *Q. palustris* Moench.
17. *T. caerulescens*. *Q. prinoides* L.
18. *T. caerulescens*. *Q. velutina* Lam.
19. *T. caerulescens*. *Q. velutina*.

20. *T. caerulescens.* *Q. virginiana* Mill.
21. *T. carnea* Johans. *Betula intermedia* Thomas.
22. *T. cerasi* (Fkl.) Sadeb. Obtained in 1935 from Centraalbureau voor Schimmelcultures. Originally isolated by M. Wieben. Probably from *Prunus avium* L. but host not definitely known.
23. *T. cerasi.* *P. avium.*
24. (a) *T. cerasi.* *P. avium.*
25. (a) *T. cerasi.* *P. pennsylvanica* L.
26. *T. cerasi.* *P. pennsylvanica.*
27. (a, b) *T. communis* (Sadeb.) Giesenhag. Fruits of *Prunus americana* Marsh.
28. (a) *T. communis.* Fruits *P. americana.*
29. (a) *T. communis.* Twigs *P. americana.* Formerly *T. decipiens* (Atk.) Giesenhag.
30. *T. communis.* Twigs *P. angustifolia* Marsh. Formerly *T. mirabilis* (Atk.) Giesenhag.
31. *T. communis.* Fruits *P. angustifolia.* Formerly *T. mirabilis* (Atk.) Giesenhag., var. *tortilis* Atk.
32. (a) *T. communis.* Fruits *P. angustifolia.* Formerly *T. mirabilis* var. *tortilis.*
33. (a) *T. communis.* *P. lanata* (Sudw.) Mack. and Bush.
34. *T. communis.* *P. maritima* Marsh.
35. (a) *T. communis.* *P. nigra* Ait.
36. (a) *T. communis.* *Prunus* sp., cultivated plum. Kansas.
37. *T. communis.* *Prunus* sp., cultivated plum. Virginia.
38. *T. confusa* (Atk.) Giesenhag. *P. virginiana* L.
39. *T. confusa.* *P. virginiana* L. var. *demissa* Torr.
40. *T. dearnessii* Jenkins. *Acer rubrum* L.
41. *T. deformans* (Berk.) Tul. *Prunus persica* L.
42. (a) *T. deformans.* *P. communis* L.
43. *T. epiphylla* Sadeb. *Alnus incana* Moench.
44. (a) *T. epiphylla.* *A. incana.* Formerly *T. klebahni* Wieben.
45. (a) *T. farlowii* Sadeb. *Prunus serotina* Thrh.
46. (a) *T. farlowii.* *P. serotina.*
47. (a) *T. flavorubra* Ray. *P. pumila* L. var. *susquehanae* Jaeg.
48. *T. flavorubra.* *P. besseyi* Bailey.
49. *T. johansonii* Sadeb. *Populus tremuloides* Michx.
50. (a) *T. johansonii.* *P. tremuloides.*
51. *T. letifera* (Pk.) Sacc. *Acer spicatum* Lam.
52. *T. nana* Johans. *Betula nana* L.
53. *T. polystichi* Mix. *Polystichum acrostichoides* (Michx.) Schott.
54. *T. polystichi.* *P. acrostichoides.* Received in 1939 from W. W. Ray, and isolated by him.
55. *T. populina* Fr. *Populus nigra* L. Isolated by M. Wieben. Received in 1935 from Centraalbureau voor Schimmelcultures.

56. (a) *T. populina. P. nigra.*
57. (a) *T. populi-salicis* Mix. *P. fremontii* S. Wats.
58. (a) *T. populi-salicis. P. trichocarpa* Torr. and Gray.
59. (a) *Taphrina potentillae* (Farl.) Johans. *Potentilla arguta* Pursh.
60. *T. potentillae. P. canadensis* L. from E. M. Martin, and isolated by her. Received in 1931.
61. (a) *T. potentillae. P. canadensis.*
62. *T. pruni-subcordatae* (Zeller) Mix. *Prunus subcordata* Benth.
63. *T. purpurascens* Robins. *Rhus copallina* L. Isolated by C. L. Kramer.
64. (a, b) *T. robinsoniana* Giesenhag. *Alnus rugosa* (Du Roi) Spreng.
65. *T. robinsoniana. A. rugosa.*
66. *T. robinsoniana.* Catkin *A. serrulata* (Ait.) Willd.
67. (a) *T. robinsoniana.* Catkin *A. serrulata.* Formerly *T. rugosa* Ray.
68. *T. robinsoniana.* Catkin *A. serrulata.*
69. *T. robinsoniana.* Leaf *A. serrulata.*
70. (a) *T. sacchari* Jenkins. *Acer saccharum* Marsh.
71. *T. sacchari. A. saccharum.*
72. (a) *T. sadebeckii* Johans. *Alnus glutinosa* Gaertn.
73. (a) *T. sadebeckii. A. serrulata* (Ait.) Willd.
74. *T. tosquinetii* (Westend.) Tul. *A. glutinosa.* Isolated by M. Wieben. Received in 1935 from Centraalbureau voor Schimmelcultures.
75. (a) *T. tosquinetti. A. glutinosa.*
76. *T. ulmi* (Fkl.) Johans. *Ulmus alata* Michx.
77. *T. ulmi. U. rubra* Muhl.
78. *T. ulmi. U. rubra.*
79. (a) *T. virginica* Sadeb. *Ostrya virginiana* (Mill.) K. Koch.
80. *Cryptococcus albidus* (Saito) Skinner. Obtained from Centraalbureau voor Schimmelcultures.
81. *Torulopsis colliculosa* (Hartm.) Sacc. From C.B.S.
82. *T. dattila* (Kluyver) Lodder. From C.B.S.
83. *Cryptococcus diffluens* (Zach) Lodder and Kreger-van Rij. From C.B.S.
84. *C. laurentii* (Kuff.) Skinner var. *flavescens* (Saito) Lodder and Kreger-van Rij. From C.B.S.
85. *Candida pseudotropicalis* (Cast.) Basgal var. *lactosa* (Harrison) Diddens and Lodder. Obtained as *Torulopsis kefyr* (Beij.) Lodder from U.S.D.A. Northern Regional Res. Lab., Peoria, Ill. Their culture y-196.
86. *Cryptococcus laurentii* (Kuff.) Skinner. From C.B.S.
87. *C. luteolus* (Saito) Skinner. From U.S.D.A.N.R.R.L.
88. *C. neoformans* (Sanfelice) Vuill. From C.B.S.

89. *Candida pulcherrima* (Lindner) Windisch. Obtained from Catherine A. Roberts. Isolated by E. M. Mrak.
90. *C. pulcherrima.* From California grapes.
91. *Torulopsis rotundata* Redaelli. From C.B.S.
92. *T. sphaerica* (Hammer and Cordes) Lodder. From C.B.S.
93. *Candida utilis* (Henneberg) Lodder and Kreger-van Rij. From C.B.S.

In the experiments all cultures were made in duplicate, in 15 × 125 mm tubes, each tube containing 4.5 ml of the following medium: KH_2PO_4 2.75 gm, $MgSO_4 \cdot 7H_2O$ 1.25 gm, dextrose 10.0 gm, distilled water 1000 ml. To this was added the particular nitrogen-source.

Inorganic nitrogen compounds were used in amounts furnishing the equivalent in nitrogen to 2.00 gm KNO_3 per liter. Peptone was used in a concentration of 2.00 gm per liter. Following experiments in which KNO_3 and asparagine were used at half strength with no reduction or with an increase in growth, the organic compounds: urea, asparagine, amino acids, were used at half strength (KNO_3-equivalent 1 gm per liter). This rendered the growth-values obtained with inorganic and organic compounds not comparable. (See later discussion of amount of growth.)

To a second set of cultures in each experiment thiamine hydrochloride was added in a concentration of one part per million. Other growth-promoting substances and trace-elements were omitted from the solutions since the object was to differentiate between organisms rather than to study the nutrition of any of them. Also the fact that many of the nitrogen-bearing compounds used contained carbon was not considered important in view of the purpose of the experiments.

Experiments with the following compounds were repeated: ammonium chloride, potassium nitrate, l-arginine monohydrochloride, asparagine, dl-aspartic acid, l-glutamic acid, dl-histidine HCl. Partial repetitions of other experiments were made in all cases (except dl-threonine) where compounds were refused by particular organisms. In these partial repetitions additional isolates of each fungus were employed if any were available.

The initial hydrogen-ion concentration of the medium was in most cases in the neighborhood of pH 4.5, this having been previously found favorable for the growth of *Taphrina deformans* and *T. communis* in certain media. In a few cases the media as made up had concentrations as high as pH 4.8 and in one case (dl-histidine HCl) pH 5.60. These media were not adjusted. Certain highly acid media were adjusted by the addition of suitable amounts of K_2HPO_4 or by the substitution of that compound for KH_2PO_4. Adjustment was made to a concentration near pH 4.5.

Final hydrogen-ion concentration was recorded in each case. Usually the degree of shift in a given direction could be taken as a measurement of the amount of growth and served to substantiate the observations of growth made in other ways. Cultures were kept at room temperature and results were taken at the end of 21 days. Amount of growth obtained was estimated by visual observation of the degree of turbidity of the solution caused by the presence of the suspended cells. These observations were expressed in symbols as follows: O (no visible growth) or tr (such slight growth as might be due to impurities in the medium); + (definite but slight growth); ++ (good growth); +++ (very good growth, comparable to that obtained with potato dextrose broth).

Amount of turbidity was also measured by means of an electrophotometer. It was not found possible to correlate the readings obtained on this instrument with cell-counts made with a haemocytometer. All organisms studied resemble yeasts in that they form in culture series of attached cells or buds. These "colonies" render accurate cell counts impossible. Therefore, since the electrophotometer readings could have only relative value, they were used only to substantiate visual observation in deciding which of the above-named symbols to employ.

Amount of growth did not actually become of much significance in these experiments and will not be reported in detail. Frequently different isolates of the same fungus made different amounts of growth in the same medium and in certain repetitions of experiments the same isolate made greater or less growth on the second trial.

Some of the growth-differences obtained on repeated trials with the same fungi are shown in TABLE I.

Detailed results of the experiments showing amounts of growth obtained in each case will not be presented. The report of a single experiment will indicate the kinds of results obtained, and will also

TABLE I

RESULTS OBTAINED IN REPEATED TRIALS OF DIFFERENT COMPOUNDS WITH DIFFERENT SPECIES OF TAPHRINA

Culture	Nitrogen compound	Trial no.	Growth without thiamine	Growth with thiamine
No. 35, *T. communis*	Potassium nitrate	1	tr	+++
		2	++	++
	Sodium nitrate	1	tr	tr
		2	+	++
		3	+	+++
	dl-alpha alanine	1	++	++
		2	+++	+++
	l-glutamic acid	1	tr	+++
		2	+++	+++
No. 27, *T. communis*	Potassium nitrate	1	+	+++
		2	++	+++
		3	+	+++
	l-arginine monohydrochloride	1	+	+
		2	+	++
No. 37, *T. communis*	Potassium nitrate	1	+	+++
		2	+	+
	dl-aspartic acid	1	+	+
		2	+	++
No. 12, *T. caerulescens*	l-arginine monohydrochloride	1	+	+
		2	+	++
No. 25, *T. cerasi*	Ammonium tartrate	1	++	++
		2	+	+++

present a list of the nitrogen compounds used. This experiment is reported in TABLE II.

Aside from amount of growth, actual utilization or refusal of various nitrogen compounds seemed, in these experiments, to be characteristic of the different species and host forms. Consideration should be given, however, to the question whether different isolates of the same species behave alike. It would seem possible that biochemical variants might exist among different isolates from the same host, even among those made at the same time. Further a number of these fungi had been maintained in culture for several years. As Roberts (4) has observed sectoring, in-

TABLE II

GROWTH MADE BY CULTURE NO. 10, TAPHRINA CAERULESCENS FROM QUERCUS LAURIFOLIA, WITH VARIOUS SOURCES OF NITROGEN

Source of nitrogen	Initial pH	Without thiamine		With thiamine	
		Growth	Final pH	Growth	Final pH
Ammonium acetate	4.86	0	—	0	—
Ammonium citrate	4.70	++	4.47	+++	4.08
Ammonium chloride	4.50	+++	3.43	+++	2.50
Ammonium nitrate	4.50	+++	2.90	+++	3.22
Ammonium oxalate	5.15	++	4.40	++	4.78
Ammonium phosphate	4.38	+	3.66	++	2.94
Ammonium sulfate	4.50	0	+	0	—
Ammonium tartrate	5.10	+	4.32	+++	3.87
Calcium nitrate	4.30	tr	—	tr	—
Magnesium nitrate	4.45	tr	—	+	4.30
Potassium nitrate	4.50	tr	4.60	++	6.10
Sodium nitrate	4.40	++	3.40	++	2.90
Peptone	4.80	+	3.72	++	4.34
Urea	4.50	++		++	5.27
dl-alpha alanine	4.80	++	4.00	+	4.70
Beta alanine	4.80	0	—	0	—
l-arginine monohydrochloride	4.40	+	3.28	+++	2.62
dl-asparagine	4.52	+	3.80	+++	4.74
dl-aspartic acid	4.30	++	3.32	+++	3.40
l-cysteine hydrochloride	4.32	0	—	0	—
l-glutamic acid	4.50	+	3.30	+++	3.12
Glycine	4.60	tr	—	0	—
dl-histidine HCl	5.60	0	—	0	—
Hydroxy-l-proline	4.58	0	—	0	—
dl-isoleucine	4.60	0	—	0	—
dl-leucine	4.60	0	—	tr	—
dl-methionine	4.66	+	4.38	+	4.32
dl-beta phenylalanine	4.60	tr	—	tr	—
l-proline	4.56	+	4.60	+	4.65
dl-serine	5.30	+	4.50	+	4.62
dl-threonine	4.62	0	—	0	—
dl-valine	4.50	+	4.52	+	4.40
Potato dextrose broth		++			

volving color change, in colonies of *Taphrina deformans*, it would seem not impossible that biochemical variants might arise in cultures of this and other species.

As will appear from examination of the list of cultures given above, of the 79 species or host-forms of *Taphrina used* in all the experiments 12 are represented by two different isolates. These are: *Taphrina caerulescens* from *Quercus maxima*, *T. caerulescens* from *Q. velutina*, *T. epiphylla*, *T. farlowii*, *T. johansonii*, *T. polystichi*, *T. populina*, *T. potentillae* from *Potentilla canadensis*,

T. robinsoniana from *Alnus rugosa, T. sacchari, T. tosquinetii, T. ulmi* from *Ulmus rubra.* The following were represented by three isolates each: *T. cerasi, T. communis* from *Prunus americana, T. communis* from *P. angustifolia, T. robinsoniana* from *Alnus serrulata.* In addition, as indicated by the letters following the culture number, 26 forms were represented by additional isolates, used whenever individual experiments were repeated. Two isolates of *Candida pulcherrima* were used throughout the experiments.

For the most part there was close agreement in the performance of different isolates of the same fungi, though sometimes one isolate made more growth than another with a particular compound. Exceptions were as follows: 1. With ammonium tartrate two cultures of *Taphrina robinsoniana* (No. 64 and No. 65) made slight (+) and moderate (++) growth respectively, while other cultures of this fungus (Nos. 66–69) made no growth (O) or a trace of growth (tr). 2. With dl-methionine, *Taphrina communis,* cultures No. 28 and No. 29, made only a trace of growth (tr) while all other isolates (No. 27, Nos. 30–37) made slight (+) but definite growth. 3. *T. communis,* culture No. 27, made slight growth with dl-leucine but a second isolate (No. 27a) of this fungus and all other isolates of *T. communis* (Nos. 28–37) refused this compound.

A special experiment was run to test the behavior of different isolates. The following nitrogen compounds were employed: ammonium chloride, ammonium phosphate and glycine. The fungi were: *Taphrina communis,* five isolates; *T. deformans,* five isolates; *T. robinsoniana* from *Alnus rugosa,* five isolates, the same fungus from *A. serrulata,* five isolates. Very close agreement in behavior was found between the different isolates. *Taphrina communis* made good use of ammonium chloride and glycine but slight use of ammonium phosphate; *T. deformans* used ammonium chloride and glycine slightly, and refused ammonium phosphate; *T. robinsoniana* from *Alnus rugosa* and from *A. serrulata* agreed in making good use of ammonium chloride and glycine, and in refusing ammonium phosphate.

All species and host-forms of *Taphrina* included in the experiments refused ammonium acetate and all of the non-sporulating yeasts but *Torulopsis dattila* used that compound. (This experiment was not repeated.) All of the fungi made good use of urea and asparagine, and all but one grew well with peptone. Therefore in reporting the behavior of the fungi in detail ammonium acetate, urea, peptone and asparagine will not be listed.

The following is a summary of the behavior of the fungi tested:

T. americana, culture No. 1, used: all ammonium compounds but ammonium chloride; calcium, magnesium, and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-cysteine hydrochloride, l-glutamic acid, glycine, dl-histidine HCl, dl-methionine, dl-beta phenyl alanine, l-proline. Refused peptone.

T. betulina, culture No. 2, used: all ammonium compounds; magnesium, potassium and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, hydroxy-l-proline, dl-isoleucine (slightly), dl-leucine, dl-methionine, dl-beta phenylalanine (slightly), l-proline (slightly), dl-serine, dl-valine.

T. betulina (*"T. lapponica"*), culture No. 3, used: all ammonium compounds; magnesium, potassium, and sodium nitrates; l-arginine monohydrochloride, l-glutamic acid, glycine (slightly), dl-histidine HCl, dl-leucine, dl-methionine, dl-serine, dl-valine.

T. betulina (*"T. turgida"*), culture No. 4, used: ammonium chloride, citrate, nitrate, oxalate; all nitrates; l-arginine monohydrochloride, l-glutamic acid, glycine (slightly), dl-histidine HCl, dl-leucine, dl-methionine, dl-serine, dl-valine.

T. caerulescens from *Quercus alba*, culture No. 5, used: all ammonium compounds but ammonium tartrate; calcium nitrate (slightly), potassium and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, glycine (slightly), l-glutamic acid, dl-histidine HCl, hydroxy-l-proline, dl-methionine, l-proline, dl-valine.

T. caerulescens from *Q. bushii*, culture No. 6, used: all ammonium compounds; all nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine

HCl, hydroxy-l-proline, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. caerulescens from *Q. coccinea,* culture No. 7, used: all ammonium compounds; all nitrates; l-arginine monohydrochloride, dl-aspartic acid, l-cysteine hydrochloride, l-glutamic acid, dl-histidine HCl, hydroxy-l-proline, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. caerulescens from *Q. ilicifolia,* culture No. 8, used: all ammonium compounds; calcium, potassium, and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, hydroxy-l-proline, dl-leucine (slightly), dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-threonine, dl-valine.

T. caerulescens from *Q. kelloggii,* culture No. 9, used: all ammonium compounds; magnesium, potassium and sodium nitrates; l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, dl-histidine HCl, hydroxy-l-proline, dl-methionine, l-proline, dl-serine, dl-valine.

T. caerulescens from *Q. laurifolia,* culture No. 10, used: all ammonium compounds but ammonium sulfate; magnesium nitrate (slightly), potassium and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, dl-methionine, l-proline, dl-serine, dl-valine.

T. caerulescens from *Q. macrocarpa,* culture No. 11, used: ammonium citrate, nitrate, oxalate; l-arginine monohydrochloride, dl-aspartic acid, l-cysteine hydrochloride, l-glutamic acid, dl-histidine HCl.

T. caerulescens from *Q. marilandica,* culture No. 12, used: all ammonium compounds; all nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, hydroxy-l-proline, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. caerulescens from *Q. maxima,* cultures No. 13 and 14, used: all ammonium compounds; all nitrates; dl-alpha alanine (slightly), l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine (slightly), dl-histidine HCl, hydroxy-l-proline, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. caerulescens from *Q. nigra,* culture No. 15, used: all ammonium compounds; potassium and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine (slightly), dl-histidine HCl, dl-leucine, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. caerulescens from *Q. palustris,* culture No. 16, used: all ammonium compounds; magnesium and potassium nitrates; sodium nitrate (slightly), l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, dl-histidine HCl, hydroxy-l-proline, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. caerulescens from *Q. prinoides,* culture No. 17, used: ammonium citrate, nitrate, oxalate; l-arginine monohydrochloride, dl-aspartic acid, l-cysteine hydrochloride, l-glutamic acid, dl-histidine HCl, dl-beta phenylalanine, dl-serine, dl-valine.

T. caerulescens from *Q. velutina,* cultures No. 18 and No. 19, used: all ammonium compounds; all nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-cysteine hydrochloride (slightly), l-glutamic acid, glycine, dl-histidine HCl, hydroxy-l-proline, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. caerulescens from *Q. virginiana,* culture No. 20, used: all ammonium compounds; magnesium, potassium, and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, dl-histidine HCl, dl-methionine, l-proline, dl-serine, dl-valine.

T. carnea, culture No. 21, used: all ammonium compounds but ammonium phosphate; all nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, dl-histidine HCl, dl-leucine, dl-methionine, dl-serine, dl-valine.

T. cerasi, cultures No. 22, No. 23, and No. 24, used: all ammonium compounds; all nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, dl-histidine HCl, hydroxy-l-proline, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. cerasi from *Prunus pennsylvanica,* cultures No. 25 and No. 26, used: all ammonium compounds; all nitrates; dl-alpha alanine, beta alanine, l-arginine monohydrochloride, dl-aspartic acid, l-

glutamic acid, glycine (slightly), dl-histidine HCl, dl-leucine, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. communis from fruits of *Prunus americana,* cultures No. 27 and No. 28, and from twigs of *P. americana,* culture No. 29, used: all ammonium compounds; all nitrates; dl-alpha alanine, beta alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, dl-beta phenylalanine, l-proline, dl-serine, dl-valine. Culture No. 27 made slight use of dl-leucine and dl-methionine.

T. communis from twigs of *P. angustifolia* (*"T. mirabilis"*), culture No. 30, and from fruits of *P. angustifolia* (*"T. mirabilis* var. *tortilis"*), cultures No. 31 and No. 32, used: all ammonium compounds; all nitrates; dl-alpha alanine, beta alanine, l-arginine monohydrochloride, dl-aspartic acid, l-cysteine hydrochloride, l-glutamic acid, glycine, dl-histidine HCl, dl-isoleucine (slightly), dl-methionine, dl-beta phenylalanine, dl-serine, dl-valine.

T. communis from *P. lanata,* culture No. 33, used: all ammonium compounds; all nitrates; dl-alpha alanine, beta alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, dl-methionine, dl-beta phenylalanine (slightly), l-proline, dl-serine, dl-valine.

T. communis from *P. maritima,* culture No. 34, used the same series of compounds as the preceding and, in addition, hydroxy-l-proline.

T. communis from *P. nigra,* culture No. 35, used the same series of compounds as culture No. 33.

T. communis from *Prunus* sp., cultures No. 36 and No. 37, used: all ammonium compounds; all nitrates; dl-alpha alanine, beta alanine, l-arginine monohydrochloride, dl-aspartic acid, l-cysteine hydrochloride (slightly), l-glutamic acid, glycine, dl-histidine HCl, dl-leucine (slightly), dl-methionine, dl-beta phenylalanine, dl-serine, dl-valine.

T. confusa from *P. virginiana,* culture No. 38, used: all ammonium compounds; all nitrates: dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine (slightly), dl-beta phenylalanine (slightly), dl-serine, dl-valine.

T. confusa from *P. dimissa,* culture No. 39, used: all ammonium compounds; calcium nitrate (slightly), magnesium, and potassium nitrates; dl-alpha alanine, beta alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, dl-isoleucine, dl-leucine, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. dearnessii, culture No. 40, used all ammonium compounds; magnesium, potassium, and sodium nitrates; dl-alpha alanine, dl-aspartic acid, l-glutamic acid, dl-histidine HCl, dl-methionine, l-proline, dl-serine, dl-valine.

T. deformans from *Prunus persica,* culture No. 41, and from *P. communis,* culture No. 42, used: all ammonium compounds but ammonium phosphate; potassium and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, l-glutamic acid, glycine (slightly), dl-histidine HCl (slightly), dl-methionine, dl-valine.

T. epiphylla, cultures No. 43 and No. 44 ("*T. klebahni*"), used ammonium chloride, citrate, nitrate, oxalate; magnesium, potassium, and sodium nitrates; l-glutamic acid (slightly), dl-histidine HCl, dl-leucine, dl-valine.

T. farlowii, cultures No. 45 and No. 46, used ammonium chloride, citrate, nitrate, oxalate, sulfate (slightly); all nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. flavorubra from *Prunus pumila* var. *susquehanae,* culture No. 47, used all ammonium compounds but ammonium phosphate; calcium, potassium, and sodium nitrates; beta alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, dl-isoleucine, dl-leucine, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. flavorubra from *P. besseyi,* culture No. 48, agreed with the preceding except for the use of magnesium nitrate and very slight use of beta alanine.

T. johansonii, cultures No. 49 and No. 50, used; all ammonium compounds but ammonium phosphate; all nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid (slightly), l-glutamic acid, glycine, dl-histidine HCl, dl-methionine, l-proline, dl-serine, dl-valine.

T. letifera, culture No. 51, used: ammonium chloride, citrate, nitrate, oxalate, phosphate (slightly); magnesium, potassium, and sodium nitrates; l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, dl-histidine HCl, dl-beta phenylalanine (slightly), dl-serine, dl-valine.

T. nana, culture No. 52, used: ammonium chloride (slightly), citrate, oxalate; magnesium, potassium, and sodium nitrates; l-arginine monohydrochloride, dl-aspartic acid (slightly), dl-histidine HCl, dl-leucine (slightly), dl-methionine (slightly), dl-valine. This culture made only slight use of asparagine.

T. polystichi, cultures No. 53 and No. 54, used: ammonium chloride, citrate, nitrate, oxalate, sulfate; magnesium, potassium, and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride (slightly), dl-aspartic acid (slightly), l-glutamic acid, dl-histidine HCl, dl-valine (slightly).

T. populina, cultures No. 55 and No. 56, used: ammonium chloride, citrate, nitrate, oxalate, phosphate; magnesium, potassium, and sodium nitrates; dl-aspartic acid, dl-methionine (slightly), l-proline, dl-valine.

T. populi-salicis from *Populus fremontii,* culture No. 57, used: all ammonium compounds; potassium and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, l-glutamic acid, dl-histidine HCl, l-proline, dl-valine.

T. populi-salicis from *P. trichocarpa,* culture No. 58, used: all ammonium compounds; all nitrates; dl-alpha alanine (slightly), l-arginine monohydrochloride (slightly), l-glutamic acid, dl-histidine HCl (slightly), l-proline, dl-valine.

T. potentillae from *Potentilla arguta,* culture No. 59, used: all ammonium compounds; all nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, dl-histidine HCl, dl-isoleucine (slightly), dl-leucine, l-proline (slightly), dl-serine, dl-valine.

T. potentillae from *P. canadensis,* cultures No. 60 and No. 61, used; all ammonium compounds; all nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, dl-isoleucine (slightly), dl-methionine, l-proline (slightly), dl-serine, dl-valine.

T. pruni-subcordatae, culture No. 62, used: all ammonium compounds; calcium, potassium, and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-histidine HCl.

T. purpurascens, culture No. 63, used all compounds but calcium, magnesium, and potassium nitrates.

T. robinsoniana from *Alnus rugosa,* cultures No. 64 and No. 65, used: all ammonium compounds (ammonium phosphate and tartrate slightly); all nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, hydroxy-l-proline, dl-leucine (slightly), dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-valine.

T. robinsoniana from *A. serrulata,* cultures No. 66, No. 67, No. 68, and No. 69, used the same compounds as the preceding.

T. sacchari, cultures No. 70 and No. 71, used: all ammonium compounds; magnesium, potassium, and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, dl-histidine HCl, dl-methionine, l-proline, dl-serine, dl-valine.

T. sadebeckii, cultures No. 72 and No. 73, used: ammonium chloride, citrate, nitrate, oxalate, phosphate, sulfate (slightly); magnesium, potassium, and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, hydroxy-l-proline, dl-leucine (slightly), dl-methionine, dl-beta phenylalanine (slightly), l-proline, dl-valine.

T. tosquinetii, cultures No. 74 and No. 75, used: all ammonium compounds; potassium nitrate; l-arginine monohydrochloride, l-glutamic acid, dl-histidine HCl, dl-leucine (slightly), dl-serine (slightly), dl-valine.

T. ulmi from *Ulmus alata,* culture No. 76, used: ammonium chloride, citrate, nitrate, oxalate, tartrate; all nitrates; l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, dl-histidine HCl, hydroxy-l-proline, dl-beta phenylalanine, dl-serine, dl-valine.

T. ulmi from *U. rubra,* cultures No. 77 and 78, used: ammonium chloride, citrate, nitrate (slightly), oxalate, sulfate (slightly), tartrate; magnesium and sodium nitrates; dl-alpha alanine, l-arginine monohydrochloride, l-glutamic acid, dl-histidine HCl, hydroxy-l-proline, dl-beta phenylalanine, l-proline, dl-valine.

T. virginica, culture No. 79, used all compounds but l-cysteine hydrochloride, dl-isoleucine, dl-leucine, dl-threonine.

Crytococcus albida, culture No. 80, used all compounds.

Torulopsis colliculosa, culture No. 81, used all compounds.

Torulopsis dattila, culture No. 82, used ammonium oxalate (slightly); l-glutamic acid, hydroxy-l-proline, dl-serine, dl-valine. This fungus refused ammonium acetate and made only slight use of asparagine.

Cryptococcus diffluens, culture No. 83, used all compounds.

Cryptococcus laurentii var. *flavescens,* culture No. 84, used all compounds but calcium nitrate.

Candida pseudotropicalis var. *lactosa,* culture No. 85, used: ammonium chloride, nitrate (slightly), oxalate, sulfate, tartrate (slightly); dl-alpha alanine, dl-histidine HCl, dl-beta phenylalanine, dl-serine, dl-valine.

Cryptococcus laurentii, culture No. 86, used all compounds but calcium nitrate.

Cryptococcus luteolus, culture No. 87, used all compounds but calcium nitrate and magnesium nitrate.

Cryptococcus neoformans, culture No. 88, used all compounds.

Candida pulcherrima, cultures No. 89 and No. 90, used all compounds but sodium nitrate and glycine.

Torulopsis rotundata, culture No. 91, used all compounds.

Torulopsis sphaerica, culture No. 92, used: all ammonium compounds; potassium nitrate; dl-alpha alanine, l-arginine monohydrochloride, dl-aspartic acid, l-glutamic acid, glycine, dl-histidine HCl, hydroxy-l-proline, dl-leucine, dl-methionine, dl-beta phenylalanine, l-proline, dl-serine, dl-threonine (slightly), dl-valine.

Candida utilis, culture No. 93, used all compounds.[2]

Certain refusals are worth mentioning. *Taphrina americana, T. caerulescens* from *Q. macrocarpa,* and *T. caerulescens* from *Q. prinoides* refused ammonium chloride, and *T. nana* used it only

[2] Four other fungi were included in the experiments. They were obtained by streak-isolation from material infected with *Taphrina aesculi, T. californica, T. carnea* (*Betula lutea*) and *T. thomasii.* They resembled known species of *Taphrina* in cellular morphology and in colony characters but they utilized all or nearly all of the compounds offered them. For the present they are believed to be unidentified asporogenous yeasts.

slightly. In a second experiment in which one-half percent agar was included in the medium, ammonium chloride was refused by seven isolates of *T. americana* (an eighth isolated made very slight growth), by eight isolates of *T. caerulescens* from *Q. macrocarpa*, and by six isolates of *T. caerulescens* from *Q. prinoides*. *T. nana* made slight growth (between tr and +).

Ammonium nitrate was refused by *Taphrina nana*, *T. ulmi* from *Ulmus rubra*, *Candida pseudotropicalis* var. *lactosa*, *and Torulopsis sphaerica*.

Of the compounds used less frequently, dl-threonine was utilized only by *T. purpurascens;* beta alanine by *T. communis*, *T. flavorubra*, and *T. purpurascens;* l-cysteine hydrochloride by *T. caerulescens* from *Quercus coccinea*, *Q. macrocarpa*, *Q. prinoides*, *Q. velutina* and by *T. purpurascens*. The compounds just mentioned were used by several of the asporogenous yeasts.

The fact that some fungi refused certain nitrates and used others, although confirmed by repetition of experiments, should, perhaps, not be emphasized. Each nitrate was added to the basal solution without the adjustment necessary to maintain the ion balance. The solution containing magnesium nitrate, for example, was richer in magnesium and poorer in potassium than the one containing potassium nitrate. To the extent that these results furnish information about the nutrition of these fungi it may be said that all organisms but *Taphrina caerulescens* from *Quercus prinoides* and from *Q. macrocarpa* are capable of utilizing nitrate nitrogen. Nevertheless the refusals observed seem to be genuine under the conditions of the experiments and are of diagnostic value.

In applying these data to the characterisation of species of *Taphrina* the following observations may be presented:

All species possess a characteristic pattern of utilization of the various compounds. This is as true for different host-forms of *Taphrina caerulescens* as it is for those fungi recognized as separate species. Previous reports by Thompson (6) and by Mix (3) have shown that all host-forms of *T. caerulescens* are different morphological entities. The host-forms included in these studies are also different physiologically.

The following host-forms of the same species were found to agree in their utilization of nitrogen compounds: *Taphrina deformans* from *Prunus communis* and from *P. persica; T. robinsoniana* from *Alnus rugosa* and from *A. serrulata; T. sadebeckii* from *Alnus glutinosa* and from *A. serrulata.*

Other host-forms showed differences. *Taphrina betulina* from *Betula intermedia* used six compounds not used by the form from the same host previously known as *T. lapponica* nor by *T. betulina* from *B. pendula.* This last named form differed from the two preceding in refusing ammonium phosphate, sulfate and tartrate, and in using calcium nitrate; otherwise it agreed with *"T. lapponica."* These three forms, previously known as separate species, were all placed in *Taphrina betulina* by the writer (3) on the basis of similar morphology. The *Taphrina lapponica* of Juel had been distinguished by him because it did not form witches' brooms but affected whole shoots. The writer's observations of this form made in Lappland in 1939 have been previously reported (l.c.). It is possible to find shoots systemically invaded that seem to have been infected for several years without the formation of witches' brooms. Nevertheless it is not possible to tell whether a recently invaded shoot will develop a witches' broom or not. It is apparent that a physiological distinction exists between *Taphrina betulina* Rostr. and *T. lapponica* Juel. Since no morphological differences can be found it seems best to continue to regard them as one species.

Taphrina cerasi from *Prunus pennsylvanica* differed from *T. cerasi* from *P. avium* in refusing hydroxy-l-proline. It also used three compounds refused by the latter fungus: beta alanine, glycine (slightly) and dl-leucine. This fungus on *P. pennsylvanica* was assigned by the writer (3) to *Taphrina cerasi* with some hesitation. It shows no morphological differences from that species. A physiological difference apparently exists.

Of the several host-forms of *Taphrina communis* here studied, some that had been previously distinguished as separate species or varieties were included in this species by the writer (3) because of morphological similarities. It is now apparent that certain physiological differences exist. If the form on *Prunus americana*

is chosen for purposes of comparison these differences can be described as follows: 1. *T. communis* from *P. angustifolia* refused dl-beta phenylalanine and l-proline (used by the preceding) and used in addition l-cysteine hydrochloride and dl-isoleucine (slightly). 2. *T. communis* from *P. lanata* and *T. communis* from *P. nigra* used dl-methionine but otherwise agreed with *T. communis* from *P. americana*. 3. *T. communis* from *P. meritima* used hydroxy-l-proline and dl-methionine (refused by *T. communis* from *P. americana*). 4. *T. communis* from *Prunus* used dl-leucine (refused by *T. communis* from *P. americana*).

Taphrina confusa from *Prunus demissa* used six compounds that were refused by *T. confusa* from *P. virginiana:* beta alanine, dl-histidine HCl, dl-isoleucine, dl-leucine, dl-methionine, l-proline. This is the greatest difference between supposedly related host-forms found in these studies.

Taphrina epiphylla and the form previously known as *T. klebahni* Wieben but placed in *T. epiphylla* by the writer (3) agreed in their behavior.

Taphrina flavorubra from *Prunus besseyi* and from *P. pumila* var. *susquehanae* agreed except that the former used magnesium nitrate and made very slight use of beta alanine.

Taphrina populi-salicis from *Populus fremontii* and from *P. trichocarpa* agreed except for refusal by the latter of calcium and magnesium nitrates.

Taphrina potentillae from *Potentilla canadensis* differed from *T. potentillae* from *P. arguta* in refusing dl-leucine and in using glycine and dl-methionine.

Taphrina ulmi from *Ulmus rubra* differed from *T. ulmi* from *U. alata* in refusing calcium and potassium nitrates, dl-aspartic acid, and dl-serine, and in utilizing two compounds refused by the former: dl-alpha alanine and l-proline.

The three species from *Acer: T. dearnessii, T. letifera* and *T. sacchari,* appear to be distinct physiological entities. Two of these, *T. dearnessii* and *T. letifera,* though occurring on different hosts are morphologically much alike (3).

Of the thirteen species of asporogenous yeasts studied six appear to be omnivorous and cannot be distinguished on the basis of

these experiments. The others showed some degree of selectivity and two of them, *Torulopsis dattila* and *Candida pseudotropicalis* var. *lactosa,* appear to be quite as specialized in their nitrogen-nutrition as many species of *Taphrina.*

Twelve of the asporogenous yeasts used ammonium acetate. Since the medium invariably became less acid during growth it is believed that the carbon of the ammonium acetate was being utilized. All species of *Taphrina* and one yeast, *Torulopsis dattila,* refused this compound.

None of the species or host-forms of *Taphrina* here studied appeared to need the addition of thiamine in order to grow in the solutions employed. In most cases, however, the addition of thiamine resulted in increased growth, and this increase was often considerable. In the case of the amino acids which were not well utilized no increase was evident, perhaps because the method of measuring growth was not sufficiently accurate. Six organisms: *Taphrina confusa* from *Prunus virginiana* var. *demissa, T. nana, T. populina, T. populi-salicis* from *Populus trichocarpa, T. prunisubeordalas* and *T. sadebeckii,* showed no benefit from the addition of thiamine to any of the solutions in which they were grown.

SUMMARY

Fifty-seven host-forms, representing twenty-seven species of *Taphrina,* and thirteen asporogenous yeasts were grown in liquid media with different sources of nitrogen as follows: eight ammonium compounds, four nitrates, urea, peptone, asparagine, and seventeen amino acids.

Ammonium chloride was refused by *Taphrina americana* and by *T. caerulescens* from *Quercus macrocarpa* and *Q. prinoides.*

Ammonium nitrates was refused by *Taphrina nana, T. ulmi* from *Ulmus rubra, Candida pseudotropicalis* var. *lactose* and by *Torulopsis sphaerica.*

Urea and asparagine were used by all the fungi studied. Peptone was refused only by *Taphrina americana.*

All non-sporulating yeasts but *Torulopsis dattila* used ammonium acetate; none of the species of *Taphrina* studied used this compound.

All species of *Taphrina* showed a characteristic pattern of nitrogen utilization. Some host-forms within species agreed closely in behavior while others showed various degrees of difference.

None of the fungi studied required the addition of thiamine to the media used, though in general they made better growth with added thiamine.

Most of the asporogenous yeasts were omnivorous or nearly so with regard to the compounds offered them. *Torulopsis dattila* and *Candida pseudotropicalis* var. *lactosa* were highly selective.

It seems possible to differentiate species of *Taphrina* in culture on the basis of their use or refusal of different compounds of nitrogen.

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THE ASCOSPORIC STAGE OF ASPERGILLUS CITRISPORUS AND RELATED FORMS

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(WITH 5 FIGURES)

An ascosporic stage of certain molds now recognized as species of *Aspergillus* has been known since 1809. In that year Link described as *Eurotium herbariorum* the yellow perithecia commonly encountered on improperly dried herbarium material. Concurrently, he described as *Aspergillus glaucus* the greenish mold (conidial stage) which occurred with these structures. Nearly a half century later, De Bary (**1854**) demonstrated that the *Aspergillus* heads and the *Eurotium* perithecia were borne on a common mycelium, thus relating Link's ascosporic structures to a large group of saprophytic molds for which no sexual stage was known In the years that followed, several additional ascosporic forms with yellow perithecia were described, usually as species of *Eurotium.* Thom and Church (**1926**) grouped these obviously related forms together as the *Aspergillus glaucus* group, an arrangement which has since become generally accepted. Meanwhile, Eidam (**1883**) described *Sterigmatocystis nidulans,* characterized by reddish ascospores, which has become the focal point for recognizing a second group of ascosporic species around *Aspergillus nidulans* (Eidam) Winter. Finally, Wehmer (**1907**) described *A. fischeri,* ascosporic but without doubt closely related to the well-defined species *A. fumigatus* Fresenius. Prior to the current report, all ascosporic molds that produce an *Aspergillus*-type of conidial stage have been referred satisfactorily to one or

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the other of these three groups. It now becomes necessary to recognize as potentially ascosporic that section of the genus to which *A. citrisporus* von Höhnel belongs.

In July 1950, and again in 1951, one of the authors (H.D.T.) isolated from Wisconsin soils an ascosporic *Aspergillus* which produces conidial heads of the general type seen in the *A. tamarii* and *A. flavus-oryzae* groups, and perithecia which are at first parenchymatous and resemble developing sclerotia but subsequently become filled with large and conspicuously sculptured ascospores. Ascocarps of this type are markedly different from those produced in the *A. glaucus, A. nidulans,* or *A. fumigatus* groups. It seemed obvious that we were dealing with an undescribed species belonging to some group wherein ascospores had not been reported previously.

In checking carefully the species recognized by Thom and Church (**1921, 1926**), reference was found to a culture obtained by them from Thaxter under the unpublished name *Aspergillus chrysospermus* which he had verbally reported to produce ascospores. Insofar as we have been able to determine, this observation was never reported in the literature; and no description of the species was ever published. In their publication of 1921, Thom and Church report "sclerotia occasionally produced" when the mold was recultivated in their laboratory. Essentially the same statement was repeated in "The Aspergilli," published five years later. Yet, in Thom's culture notes dated May 20, 1919, the following notations appear with reference to Thaxter's culture: "Perithecia, 500 μ—soft, easily crushed. Asci 12–14 μ diam." Ascospores were not observed.

Upon the basis of their examination of the conidial structures of Thaxter's fungus and von Höhnel's brief, but seemingly well-prepared description of *A. citrisporus,* Thom and Church concluded that the fungi observed by von Höhnel and Thaxter represented a single species. Accordingly, they accepted von Höhnel's usage, recognized *A. citrisporus* as the valid species name, and distributed Thaxter's isolate to various laboratories, including the Centraalbureau in Baarn, as *A. citrisporus,* strain No. *4186.10.*

When cultivated at the Northern Regional Laboratory and compared with known species of the *A. tamarii* group, the similarity between Tresner's isolates and Thom and Church's concept of *A. citrisporus* became clearly evident. We then obtained Thaxter's exsiccati (labeled *A. chrysospermus*) from the late Professor W. Lawrence White, Curator of the Farlow Herbarium, in order to compare the ascospores of his material with those produced by the new isolates from Wisconsin soil. Also, we secured from the Centraalbureau, through the courtesy of Dr. Johanna Westerdijk, the culture of *A. citrisporus* which had been deposited by Thom and Church in March 1925 and which stemmed originally from Thaxter's laboratory. In our hands this latter culture has failed to produce ascospores, although a great variety of culture media has been investigated. Fortunately, we found abundant perithecia and ascospores in Thaxter's herbarium specimens. In this material, as in the growing cultures of Tresner's isolates, the perithecia were characterized by a marked purplish coloration and superficially resembled the sclerotia formed in many species of *Aspergillus*. The ascospores, however, were strikingly different from those produced in the Wisconsin isolates. This circumstance, together with certain other differences of lesser significance, has led us to conclude that two species are involved, and that each merits recognition as an ascosporic species. This necessitates (1) the redescription of *A. citrisporus* to include a perfect stage, exemplified by Thaxter's exsiccati and the still living culture (conidial phase only) which stems therefrom, and (2) the description of a new species, *Aspergillus ornatus,* to include the Wisconsin strains.

SPECIES RECOGNIZED

ASPERGILLUS CITRISPORUS von Höhnel emend.

Colonies on Czapek's solution agar restricted and thin, attaining a diameter of 1.0 cm in two weeks at room temperature (FIG. 1A), consisting of a sparse, submerged growth of vegetative hyphae, and bearing few small conidial structures with conidiophores seldom exceeding 500 μ in length or vesicles 15 μ in diameter.

Colonies on malt extract agar growing rapidly, attaining a diameter of 8–10 cm in 10 days at room temperature, confluent, heavily

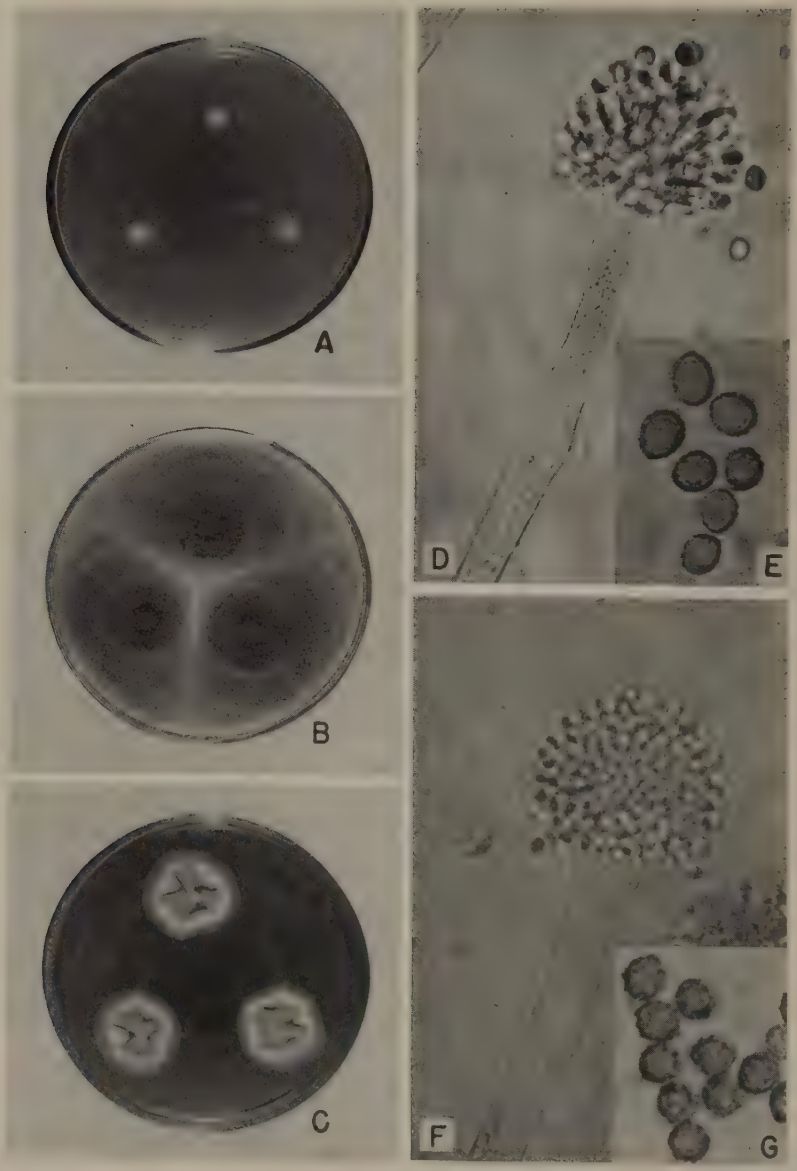

FIG. 1. *Aspergillus citrisporus.* Conidial phase. A, B, and C. Cultures growing on Czapek's solution agar (with sucrose), malt extract agar, and Czapek's agar with glucose, respectively. 10 days incubation in diffuse daylight at room temperature. D. Conidial head of NRRL *2292*, which strain stems from one of Thaxter's collections (see FIG. 2 and text) through Thom's No. *4186.10*, × 450. E. Mature conidia of the same, × 1000. F. Conidial head of NRRL *2293* (Warcup's isolate), × 450. G. Mature conidia of the same strain, × 1000.

and uniformly sporulating throughout with conidial structures arising from submerged vegetative mycelia to form a dense stand (FIG. 1B); incubation in darkness not restrictive; conidial heads in orange-yellow shades, at first near aniline yellow, becoming orange citrine at maturity, and darkening to medal bronze in age (Ridgway, IV); reverse uncolored; odor pronounced, somewhat foetid. Conidial heads radiate, mostly 150–200 μ, but ranging from 100 to 225 μ, with conidial chains loosely divergent. Conidiophores variable, commonly 1.5–2.0 mm in length by 15–20 μ in diameter above, septate, with walls comparatively thick (*ca.* 1.0–1.5 μ at median level), smooth as seen in fluid mounts but appearing granular when observed dry. Vesicles somewhat flask-shaped, 20–30 μ diameter with uppermost three-fourths or entire surface bearing sterigmata in a single series (FIG. 1D), often loosely arranged. Sterigmata variable, generally 8–12 × 5.5–7.0 μ. Conidia citriform or pyriform to subglobose, light yellow-brown, conspicuously roughened, mostly 6.0–7.5 × 5.0–6.0 μ (FIG. 1E).

Colonies on potato dextrose agar essentially like those produced on malt extract agar but growing somewhat more thinly and producing a less dense stand of conidiophores.

Colonies on Czapek's solution agar containing glucose instead of sucrose growing somewhat restricted (FIG. 1C), forming a dense mycelial felt somewhat wrinkled and furrowed, and producing abundant but generally diminutive conidial structures.

Perithecia (seen in Thaxter's exsiccati only) globose or nearly so, variable in size but commonly 300–500 μ in diameter, in purple shades, with or without an enveloping network of much-branched, coarse mycelia, upon crushing appearing somewhat parenchymatous but well filled with ascospores; perithecial wall comparatively thin at maturity and composed of polyhedral cells 10–12 μ diameter (FIG. 2E); asci with nearly mature spores measure about 12 μ diameter (FIG. 2C); ascospores lenticular, smooth-walled, 7.5–8.5 × 5.0–6.0 μ, with single (?) broad equatorial bands (FIG. 2C, F).

The emended description of *Aspergillus citrisporus* von Höhnel here presented is based upon a culture, NRRL *2292,* bearing this name which was received from the Centraalbureau voor Schimmelcultures in June, 1951, together with Thaxter's herbarium specimens obtained from the Farlow Herbarium of Cryptogamic Botany, Harvard University. There is ample reason to believe that the former culture stems directly from the latter, and that we are, in fact, drawing the description of conidial and ascosporic

stages from the same original isolation, albeit the connecting links are somewhat devious and extend over more than a quarter century. The story is as follows: The culture of *A. citrisporus* received from the Centraalbureau is listed as having come from Thom and Church. Records of these latter investigators indicate that a fungus bearing the numerical designation *4186.10* was sent to Baarn in March, 1925, under this name. This culture, cited

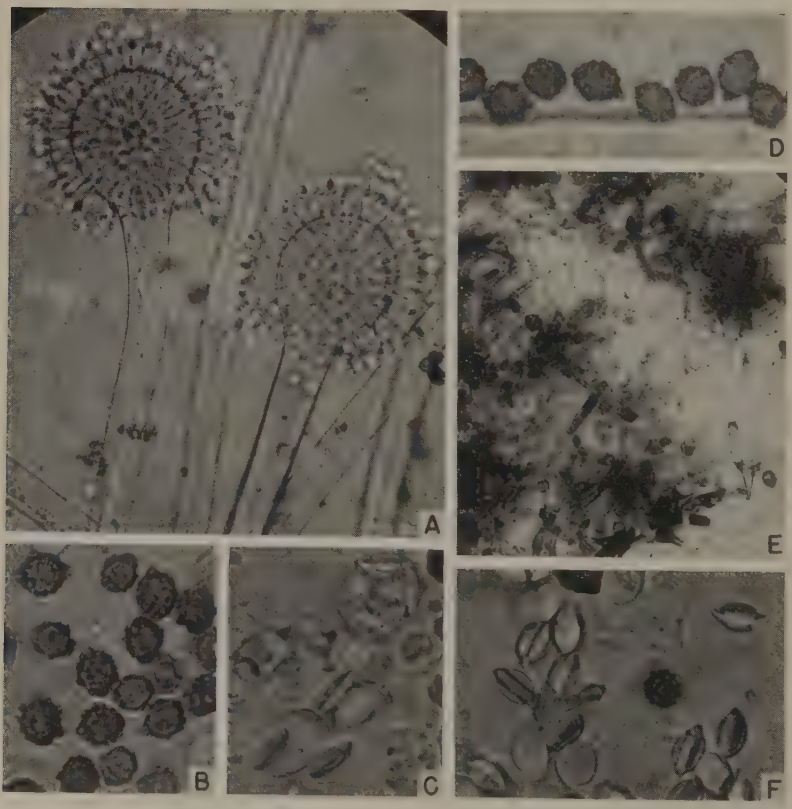

FIG. 2. *Aspergillus citrisporus;* Thaxter's herbarium material. A. Conidial structures from his dried culture (medium not stated) made from the "Chocoura, N. H." collection, × 275. B. Mature conidia from the same, × 850. C. Asci and ascospores from the same culture, × 850. D. Mature conidia from a dried culture of the "Kittery Point" collection, × 850. E. Portion of perithecial wall from same culture, showing hexagonal peridial cells and fragments of branched enveloping hyphae, × 275. F. Mature ascospores from the same, × 850.

by Thom and Church (1926) as representative of *A. citrisporus,* had been obtained from Thaxter as *"Aspergillus chrysospermus"* in March, 1917, and entered in Thom's Collection under the number indicated above. At this point an element of uncertainty enters our chain of evidence, although it in no way invalidates the claim of culture continuity from Thaxter's laboratory to our own. Among Thaxter's material are two groups of specimens, both of which (1) represent isolates from caterpillar dung; (2) bear the designation *A. chrysospermus;* (3) show conidial heads with single sterigmata (FIG. 2A) and large, coarsely roughened, citriform conidia (FIG. 2B, D); and (4) contain purple, parenchymatous perithecia with large, smooth-walled ascospores which are marked by single broad equatorial bands (FIG. 2C, F). One of the two is labeled "Chocorua, N. H., Aug., 1909" (FIG. 2A, C); the other is marked "Kittery Point" and bears no collection date (FIG. 2D, F). Whereas it is possible that the culture given to Thom in 1917 may have come from either source, it is believed probable that the Kittery Point collection is of more recent date and that NRRL *2292* stems from it. The point is not too important, in any case; for insofar as we can determine the two collections are strikingly similar.

A third collection of Thaxter's labeled "Hammond's Pond, Mass., Sept. 17, 1902," is preserved only as a permanent slide mount and shows no ascosporic stage, hence is not believed to represent a possible progenitor of NRRL *2292.* The few conidial heads that are present in this specimen, however, agree closely with the other two.

One additional culture, NRRL *2293,* which we believe to represent the species under consideration, was isolated from New Zealand forest soil and sent to us in January, 1948, by Dr. J. H. Warcup of the Botany School, Cambridge University, as *"A. citrisporus* von Höhnel (?)." This produces heavily sporulating colonies on malt extract agar and on other natural substrata of this type. The conidiophores are appreciably shorter than those cited above, but in other particulars it agrees reasonably well with the species as here described (FIG. 1F, G). In aging cultures (3–6 weeks) on malt extract agar, colonies commonly de-

velop white mycelial tufts which superficially suggest the perithecia seen in Thaxter's specimens and regularly produced in growing culture of *A. ornatus*. Upon closer examination these are found to consist of rather densely interwoven hyphal threads rather than parenchymatous tissue, and no asci or ascospores have been observed. Despite their absence, we feel that these structures probably represent abortive perithecia, and it is hoped that some combination of substratum and cultural conditions can be found which will permit their full development. At such time only can the strain in question be diagnosed more satisfactorily.

Aspergillus ornatus n. sp.

Coloniae in agaro Czapekii tarde crescentes, tenues, pellucidae, fructificationibus conidicis ex hyphis hyalinis submersis vegetis oriundis, peritheciis carentibus, in agaro maltoso luxuriantes, frutificationibus conidicis ex hyphis submersis oriundis et super stratum superficialem peritheciorum protrudentibus, late zonatae vel sectores efformantes, praecipue flavo-aurantiacae, e primulino aureae usque olivaceo-citrinae, reverso non colorato; capitulis conidicis radiatis, plerumque 300–400 μ in diam.; conidiophoris septatis 1.0–1.5 mm longis, 12–18 μ in diam.; membranis comparate crassis, humidis glabris, sed aridis subasperatis; vesiculis lageniformibus, per quartas tres superiores fertilibus, plerumque 25–40 μ in diam.; sterigmatibus uniseriatis, confertis, plerumque 8–10 $\times$ 4.5–5.5 μ; conidiis e aureis pallide brunneis, citriformibus vel pyriformibus, e liris irregularibus vel lineis coloratis crasse asperatis, plurimum 6.5–8.0 $\times$ 5.0–6.0 μ; peritheciis in agaro maltoso abundantibus, primum albis parenchymaticisque, in maturitate purpurascentibus, parte fertili peridio cellularum irregularium polyedricarum circumdata; ascis globosis, 14–15 μ; ascosporis lenticularibus, asperatis, cristis multiplicibus tenuibus 1 μ latis irregulariter sed vulgo longitudinaliter dispositis ornatis.

In culturis e solo in silvis, Wisconsin.

Colonies on Czapek's solution agar growing very sparingly, 2.0–2.5 cm in 10 days at room temperature, thin, transparent (FIG. 3A), with scattered small conidial structures arising from submerged and colorless vegetative hyphae; conidial heads radiate, commonly 150–200 μ in diameter and borne on conidiophores seldom more than 650 μ in length. No perithecia produced.

Colonies on malt extract agar growing luxuriantly, attaining a diameter of 7.0–8.0 cm in 10 days at room temperature (FIG. 3B), with vegetative mycelium largely submerged or superficial, broadly zonate from alternating bands of crowded and less numerous conidial structures projecting above the surface layer of abundant perithecia, less commonly tending to sector with some areas pre-

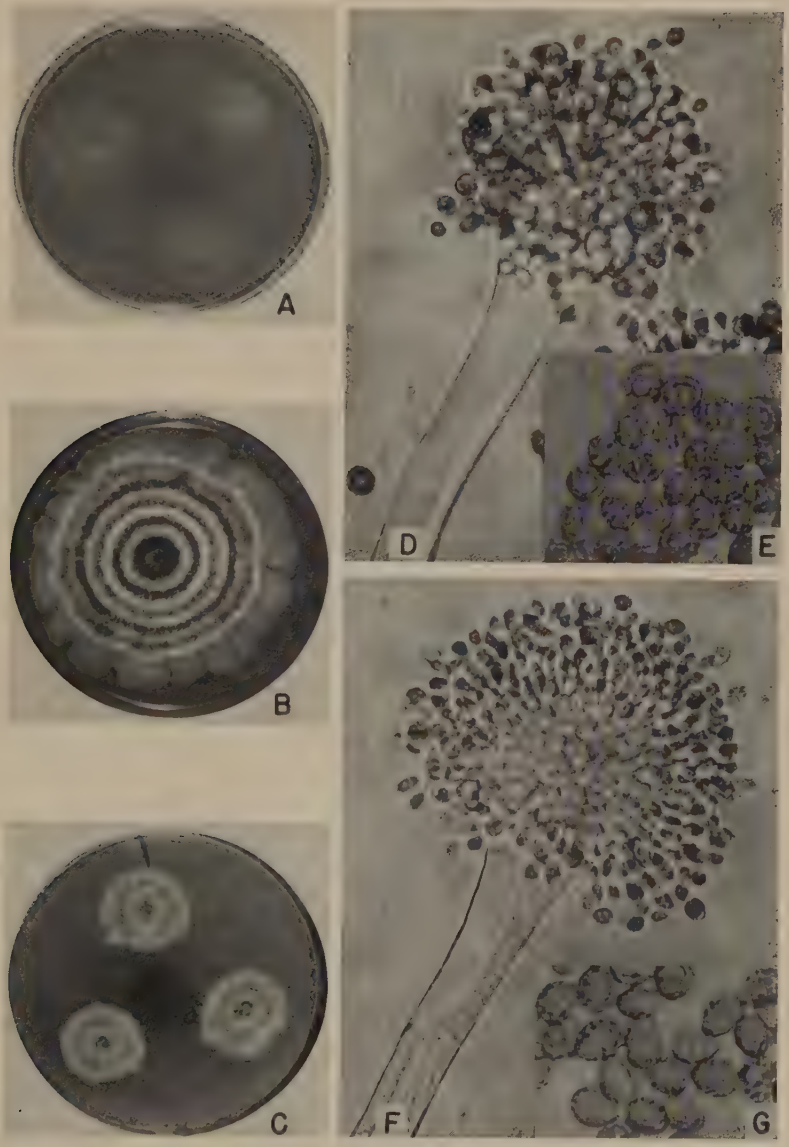

FIG. 3. *Aspergillus ornatus.* Conidial phase. A, B, and C. Cultures of type strain NRRL *2256* growing upon Czapek's solution agar (with sucrose), malt extract agar, and Czapek's agar with glucose, respectively. 10 days incubation in diffuse light at room temperature. D. Conidial head in type strain, × 525. E. Mature conidia of the same, × 1000. F. Conidial head in strain NRRL *2291* (Tresner's second isolate), × 450. G. Mature conidia of the same, × 1000.

dominantly conidial and others heavily perithecial; conidial structures arising from submerged hyphae, abundantly produced in light, lacking or sparingly developed in darkness, in yellow-orange shades from primuline yellow when young through old gold to olive-citrine in age (Ridgway, XVI); heads radiate, with conidial chains divergent and somewhat tangled, variable in size, commonly 300–400 μ in diameter but ranging from 200–450 μ; conidiophores septate, commonly 1.0–1.5 mm in length by 12–18 μ diameter above, somewhat narrowed below, with walls comparatively heavy (*ca.* 1.0–1.5 μ at a median level), appearing smooth in fluid mounts but somewhat roughened when examined dry; vesicles flask-shaped, mostly 25–40 μ diameter, fertile over the upper three-fourths, bearing crowded sterigmata in a single series; sterigmata crowded, mostly 8–10 × 4.5–5.5 μ; conidia golden to light brown, mostly citriform to pyriform, coarsely roughened from an irregular network of ridges or color bars, commonly 6.5–8.0 × 5.0–6.0 μ. Perithecia abundantly produced, variable in size but commonly 400–600 μ diameter, at first white and parenchymatous throughout, becoming purple and filled with asci and ascospores at maturity (FIG. 4C), the fertile portion remaining surrounded by a residual peridium of irregular polyhedral cells 15–20 μ in diameter (FIG. 4D) within a loose mantle of sterile, much-branched hyphae; asci globose, 14–15 μ at maturity; ascospores lenticular, rough-walled, with multiple thin, flexuous crests approx. 1.0 μ wide arranged irregularly in a generally longitudinal direction, over-all dimensions generally 7.5–8.5 × 5.5–6.0 μ (FIG. 4E).

Colonies on potato dextrose agar spreading broadly, 8–9 cm in 10 days, comparatively thin, especially in marginal areas; conidial heads abundant but not crowded, averaging about 250 μ in diameter, and near old gold (Ridgway, XVI) when mature; perithecia abundantly produced, averaging somewhat smaller than on malt extract agar.

Colonies on Czapek's solution agar with glucose instead of sucrose growing somewhat restrictedly, 2.0–2.5 cm in 10 days (FIG. 3C), with compact basal mycelium more or less wrinkled producing numerous conidial heads, thinning toward the irregular colony margin; perithecia occasionally developed in old cultures.

The binomial *Aspergillus ornatus* is chosen because of the extraordinary ornamentation of the ascospores.

Type culture NRRL *2256* was isolated by one of the authors (H.D.T.) from soil collected in an oak woods just west of the city of Madison, Dane County, Wisconsin (7N–9E–29), in July, 1950.

A second strain (NRRL *2291*), which duplicates the type in all essential particulars, was subsequently isolated from soil collected in a maple woods in Spring Grove Township, Greene County, Wisconsin (1N–9E–29). Both soils were taken from the "A"

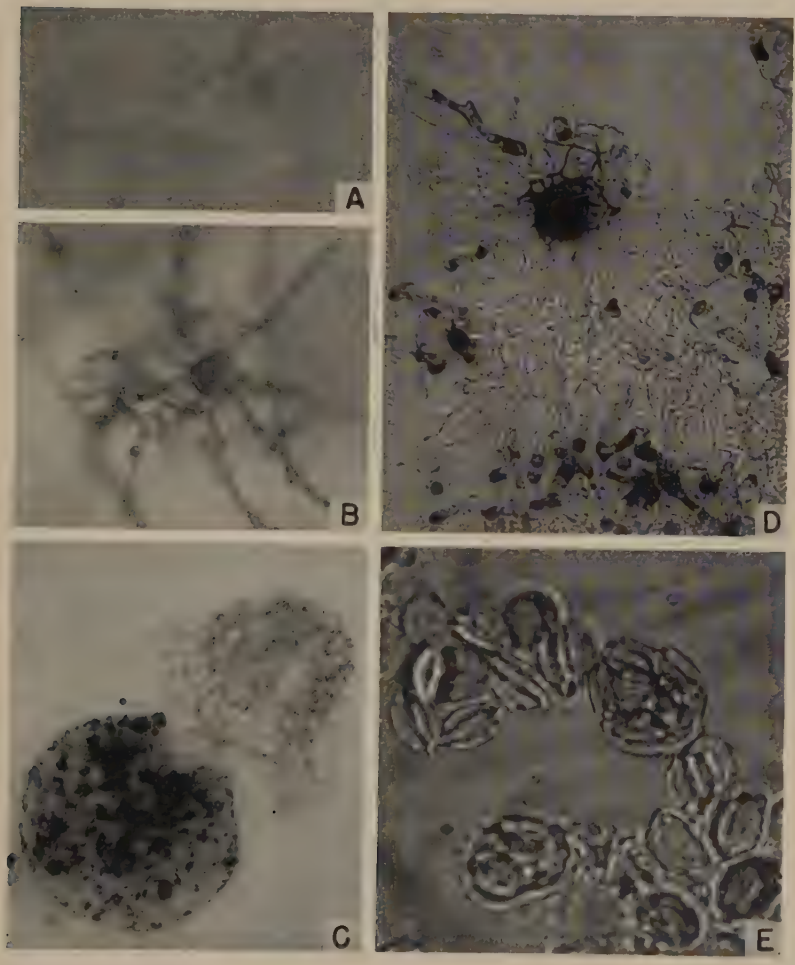

FIG. 4. *Aspergillus ornatus:* NRRL *2256*. Ascosporic phase. A. Young ascogonium, × 175. B. Somewhat later stage, × 175. C. Mature perithecium, crushed to expose ascigerous tissue, × 65. D. A portion of the perithecial wall showing the hexagonal cells and remnants of the coarse branched hyphae which comprise it, × 325. E. Mature ascospores showing characteristic ornamentation, × 1000.

horizon, i.e., the top 1½ inches. The writers are indebted to Professor Myron P. Backus, University of Wisconsin, for having made the preliminary examination of this fungus, for recognizing the unusual characteristics of its ascosporic phase, and for bringing it to our attention in order that we might study it critically.

GROUP RELATIONSHIPS

Aspergillus citrisporus von Höhnel emend. and *A. ornatus* represent a new departure in ascosporic species of *Aspergillus*. For several reasons they cannot be placed in any of the ascosporic groups previously recognized. Like members of the *A. glaucus* group, they produce conidial heads that are radiate in pattern and bear a single series of sterigmata; but, unlike these forms, they produce conidiophores which appear roughened when viewed in dry mounts. Most importantly, they develop purple, parenchymatous perithecia that are markedly different from the consistently yellow, thin-walled structures seen in *all* ascosporic species of the *A. glaucus* group. They cannot be closely related to *Aspergillus nidulans* (Eidam) Wint. and allied forms, for they do not develop Hülle cells or show colored stalk walls. Additionally, they produce colorless ascospores in contrast to the reddish spores that are so characteristic of the *A. nidulans* group. They obviously do not belong with *A. fischeri* Wehmer, for their conidial heads are much larger and are radiate rather than columnar. As with the preceding groups, the most striking difference consists in the character and texture of their ascocarps as contrasted with those of *A. fischeri*.

Whereas the origin of perithecia cannot be established for *A. citrisporus* from currently available material, in *A. ornatus* perithecia arise from coiled ascogonia (FIG. 4A, B) that are strikingly different from the initially unbranched primordia observed in other ascosporic groups. Additionally, ascospores seem not to germinate by valvular separation as in other Aspergilli, but by simple swelling and the production of germ tubes as in the conidia of this species and in the ascospores of many Penicillia.

Superficially, colonies and conidial structures of *A. citrisporus* and *A. ornatus*, when grown upon favorable media such as malt

extract agar, bear a striking resemblance to those of some members of the *A. tamarii, A. wentii,* and *A. flavus-oryzae* groups. Furthermore, immature perithecia of the two former species are strongly suggestive of the developing sclerotia that characterize the latter groups of asexual Aspergilli. Weighing such facts, together with differences in the perithecial stage of the presently reported ascosporic species as compared with perfect forms long verified, we are led to the conclusion that a fourth group must be recognized as potentially ascigerous.

It is perhaps too early to determine just what realignments should be made within the genus *Aspergillus.* Obviously, the arrangement of groups and species worked out by Thom and Church (**1926**) and, in the main, restated by Thom and Raper (**1945**), will have to be altered. The schematic diagram of group relationships presented by the latter (**1945**, p. 87) will have to be redrawn, placing greater or lesser emphasis upon some of the characters which heretofore have been used effectively to differentiate groups of species that are clearly related. Based upon present knowledge, and the comparative examination of cultures presently available, it would seem desirable to regard the *A. tamarii* group as an ascigerous one, recognizing, as we do in the *A. glaucus, A. nidulans,* and *A. fumigatus* groups, that all member species need not attain this level of development. In time it may be found desirable to transfer the species *A. tamarii* Kita to the *A. flavus-oryzae* group, with which it shares many characteristics in common, and to recognize a new group of species centering upon some ascosporic form such as *A. citrisporus* or *A. ornatus* and to include with it *A. lutescens* (Bain.) Thom and Church, *A. terricola* Marchal, and *A. terricola* var. *americana* Marchal. This would bring together a fairly homogeneous group of species characterized by loose-textured, radiate conidial heads with sterigmata in a single series, except for the larger structures in *A. lutescens.*

The precise natural substratum of *A. ornatus* is not known. In the case of *A. citrisporus,* however, all known isolates, with the exception of NRRL *2293* from Warcup, have been obtained from the dung of various insect larvae. It is easily possible that such material actually supported Warcup's isolate as well as the two

isolates of *A. ornatus*. On the other hand, all of these forms may occur occasionally in soil; and the high incidence of *A. citrisporus* on caterpillar dung may merely reflect inoculation from such source, coupled with the subsequent collection of dung pellets as discreet entities.

PHYSIOLOGICAL STUDIES

In preliminary cultural investigations performed at the University of Wisconsin it was observed that the isolate (NRRL *2256*) which now constitutes the type of *Aspergillus ornatus* grew very thinly, sporulated sparsely, and produced no perithecia upon Czapek's solution agar; whereas it grew luxuriantly and produced abundant conidial heads and perithecia on malt extract and potato-dextrose agars. This observation was immediately confirmed at the Northern Regional Research Laboratory upon receipt of the culture from Professor Backus. Furthermore, it was soon noted that in plate cultures on Czapek's solution agar, *A. ornatus* grew well and sporulated freely in portions of colonies adjacent to contaminating molds. Thus it was obvious that the culture suffered from some nutritional deficiency which was partially or wholly corrected by the contaminant.

Two possibilities immediately suggested themselves: (1) A need for some essential vitamin, or (2) an inability to utilize sucrose.

To test the first of these possibilities, vitamins of the B-complex were added in excess *via* cylinder cups to cultures of *A. ornatus* growing upon Czapek's solution agar plates. Doubtfully significant responses were obtained with riboflavin (B_2) and cobalamin (B_{12}), whereas no measurable response was obtained with thiamin, inositol, para-amino benzoic acid, pyridoxine, pyridoxal, biotin, folic acid, nicotinic acid, and pantothenic acid. Since vitamins B_2 and B_{12} were added as strongly acidic solutions (pH 2.8 and 1.5, respectively), the possible effect of pH could not be ruled out; and subsequent tests tended to minimize the possibility of a true nutritional response from either vitamin.

Meanwhile, other experiments pointed to an inability to utilize sucrose as an increasingly probable explanation. For example, it

was observed that *A. ornatus* attained satisfactory, but not luxuriant, growth on Czapek's solution agar containing glucose instead of sucrose and on Czapek's solution agar with sucrose as a carbon source but with KH_2PO_4 substituted for K_2HPO_4. Since it was known that sucrose when heat sterilized in the presence of KH_2PO_4 would be partially hydrolyzed, it was thought that the mold might be growing only upon the hexoses present in the latter medium, as it was of necessity doing in the former. By analysis, Czapek's solution agar with initial concentrations of 3% sucrose and 0.1% KH_2PO_4 showed from 0.3 to 0.4% reducing sugar and from 2.6 to 2.7% sucrose after heat sterilization. Interestingly enough, the growth of *A. ornatus* on such media was not obviously greater than on Czapek's solution agar containing 0.3% glucose as sole carbon source and 0.1% KH_2PO_4, where both the sugar and the phosphate were added as Seitz filtrates. In other experiments Seitz-filtered sucrose was added to sugar-free Czapek's solution agars of varying pH, and in no case did growth exceed a very thin, submerged mycelium bearing few and diminutive conidial heads. Obviously, sucrose could not be utilized by *A. ornatus;* but reducing sugars, particularly glucose, seemed to afford an acceptable carbon source. Further tests indicated that this constituted only a partial answer. An unanticipated response was encountered when Seitz-filtered glucose was similarly added to sugar-free agar media of different pH. Good growth was initiated rapidly in acidic media (pH 5.0–5.5), whereas for a period of several days growth was minimal on media of slightly alkaline reaction (pH 7.5 ±). By the end of two weeks, however, growth was proceeding satisfactorily in the latter, and the reaction of the substrate adjacent to the growing colonies had been depressed to pH 4.5 ±, which at this age approached the reaction attained by cultures growing in the initially acidic media. From the above, and other substantiating experiments, we conclude that glucose constitutes a favorable carbon source for *A. ornatus*. It becomes optimally effective, however, only in the presence of an acidic environment. This may be established prior to inoculation by the introduction of acid phosphate, or it may be created by the growing fungus itself by the accumulation of acidic products which

result from the metabolism of glucose. The sugar availability *vs.* acidity relationship seems to be even more striking in the case of fructose. This sugar (as a Seitz filtrate) affords a satisfactory carbon source for *A. ornatus* when added to acidic (pH 5.5 ±), carbon-free agar media. On the other hand, growth hardly exceeds that obtained with sucrose on comparable media that are at first neutral or slightly alkaline, and the pH is not significantly depressed, even after two weeks incubation.

Whereas these nutritional investigations have centered around *A. ornatus* NRRL *2256,* the other strain of this species and those of *A. citrisporus* already mentioned have been included quite often for comparison. This has been especially true of NRRL *2292,* the culture of *A. citrisporus* supplied by the Centraalbureau. Allowing for normal species and strain variation among closely related forms, these other cultures behave in a manner strikingly similar to NRRL *2256.* In every case, growth is luxuriant upon malt extract agar and is minimal upon Czapek's solution agar containing sucrose as a carbon source, irrespective of pH. In each case satisfactory growth is obtained with glucose as carbon source in media which are initially acidic. Strain NRRL *2292* differs from the others in being less able to grow satisfactorily on glucose media of initial neutral reaction even after extended incubation.

The production of conidia and perithecia by *Aspergillus ornatus* NRRL *2256* is strongly affected by light. Initial experiments were conducted in the Botany Department, University of Wisconsin, by M. Louis Bobichon and others. Concerning these studies, Professor Paul J. Allen writes as follows (personal communication):

"This [light] effect was studied particularly on [an agar] medium containing 4% of an extract of blended woody twigs, which permits growth at half the maximum rate obtained with honey-peptone agar. In complete darkness no conidia are formed on this medium, while in continuous white light of low intensity heavy sporulation occurs. Illumination by a spectrum projected across a plate which had been uniformly inoculated with spores resulted in sporulation in blue light. A few minutes' illumination of mycelium which has grown in the dark caused heavy sporula-

tion only at the margin of the colony and not on the older parts. Marked zonation is therefore exhibited by colonies grown in alternating light and dark.

"Light distinctly inhibits perithecia formation, but does not suppress it entirely."

Some additional investigations were made at the Northern Regional Laboratory. For the most part, these were comparative studies wherein strain NRRL *2256* was contrasted with the second isolate of *A. ornatus,* NRRL *2291,* and with the two strain assigned to *A. citrisporus* NRRL *2292* and *2293.* Observations

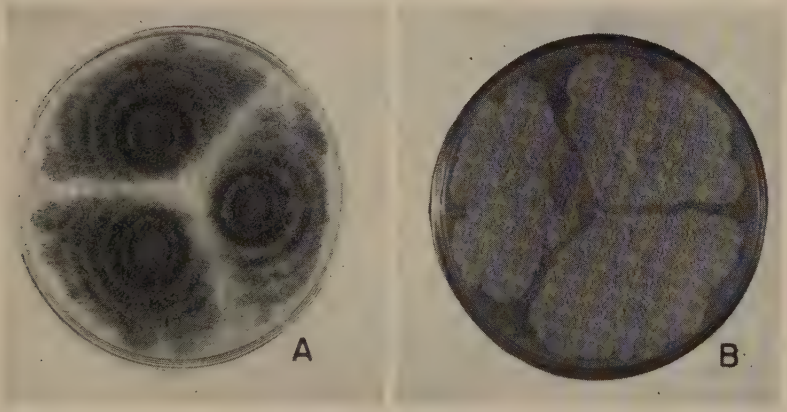

FIG. 5. Effect of light upon the production of conidial structures in *Aspergillus ornatus* NRRL *2256.* Colonies on malt extract agar, 10 days incubation at room temperature. A. Culture incubated in diffuse daylight. B. Culture incubated in darkness.

made on NRRL *2256* confirmed, in general, those reported from Wisconsin. On the majority of media investigated, colonies produced very few or no conidial heads when incubated in darkness, whereas such structures were produced abundantly in parallel cultures incubated in diffuse daylight (FIG. 5A, B). Czapek's solution agar enriched with 1% corn steep liquor provided a striking exception: On this medium conidial production in darkness approached the level attained in light. Incubation temperatures ranged between 24–27° C but were uniform for light- and dark-incubated cultures.

Aspergillus citrisporus NRRL *2292* and *2293* showed a response markedly different from *A. ornatus* NRRL *2256*. Neither of the former strains showed any apparent difference in conidial production whether the cultures were incubated in darkness or in light. *A. ornatus* NRRL *2291* was somewhat intermediate in behavior, conidial production being sharply reduced but not suppressed in dark-incubated cultures.

Obviously, the effect of light on sporulation represents a strain characteristic. Nevertheless, it may prove valuable in differentiating between *A. ornatus* and *A. citrisporus*. The observation that the effect of darkness can be tempered in NRRL *2256* by modifying the substrate suggests some interesting possibilities for further investigation. What is contained in steep liquor that enables the mold to circumvent, in large measure, the metabolic block which on other substrata effectively precludes the development of conidial structures in darkness?

DISCUSSION

A number of considerations led us to associate the Wisconsin isolates with *Aspergillus citrisporus* von Höhnel. Among these was the general aspect of growing colonies on a variety of substrata, together with the color and pattern of the conidial structures which are characterized by radiate heads bearing sterigmata in a single series. Whereas conidial heads of this structural type are regularly seen in certain members of the *Aspergillus flavus-oryzae* group, e.g., *A. parasiticus* Speare and some cultures of *A. oryzae* (Ahlburg) Cohn, a golden to light olive-brown coloration of conidial heads and the absence of consistently rough stalks in the Wisconsin strains indicated closer relationship with members of the *A. tamarii* group (see p. 683). Criteria which pointed more specifically to *A. citrisporus* were found in descriptions of this species published by Thom and Church (**1921**, **1925**), and more particularly in Thom's unpublished cultural notes. In these the meagre growth of the species on Czapek's solution agar was emphasized, and some attention was given to the "sclerotia" (perithecia) which were produced in Thaxter's culture shortly after its receipt. Altogether, the conidial phase of the Wisconsin

isolates seemed to fit reasonably well the species concept for *A. citrisporus* von Höhnel as this had been interpreted, enlarged, and presented by Thom and Church (1921).

With a feeling of confidence that our presumptive diagnosis would be confirmed, we requested Professor White to lend us Thaxter's collections representing *A. citrisporus,* or *A. chrysospermus,* the unpublished name used by Thaxter. In particular, we wished to obtain the specific collection from which Thom's No. *4186.10* stemmed, for this culture had formed the principal basis for accreditation of *A. citrisporus.* Furthermore, it had been deposited with the Centraalbureau, and for twenty-five years it had been distributed from that Collection under this name. As expected, the conidial structures in Thaxter's materials, after reconstitution, were generally similar to those in the Wisconsin strains. Furthermore, the perithecia in these dried specimens were strikingly similar in color and texture to those regularly produced in growing cultures of the new isolates. The ascospores, however, were markedly different (FIG. 2C, F, 4E). It was at first difficult to believe that the spores were not basically similar, and that apparent differences could not be attributed to thoroughly dry material in the one case as contrasted with living material in the other. The observed differences were accepted as real, however, when recognized techniques for swelling and reconstituting fungus tissues failed to reveal any method of treatment whereby the ascospores appeared similar.

One may question the wisdom of combining, in a single species description, observations taken from living cultures on the one hand and from long dried herbarium materials on the other. In the case of *A. citrisporus* we believe this to be justified, for we can trace the two contributory elements to a common source with reasonable certainty. Such treatment, of course, does not take into account possible variations in the living culture which has for a quarter of a century been grown on laboratory substrata. Furthermore, there is evidence that our accepted culture of *A. citrisporus,* NRRL *2292,* has changed somewhat during this period. The conidia would seem to be less coarsely roughened, heads and vesicles would seem to be somewhat smaller, and, of

course, the capacity to produce perithecia and ascospores has been lost. Admittedly, our approach is not entirely satisfactory. On the other hand, if we were to prepare the complete diagnosis from exsiccati, we would perforce exclude such characteristics as color, rate of growth, and texture and appearance of the growing fungus —features which are most important in identifying species of *Aspergillus*.

One may question, also, whether *A. ornatus* merits recognition as a new and separate species. Certainly, the conidial stages are strikingly similar in this species and in *A. citrisporus*, as we interpret the latter. Furthermore, the perithecia are quite similar in the two cases. Such parallelism, however, does not invalidate species recognition; in fact, the situation is strictly comparable to accepted practice in the *A. glaucus* and *A. nidulans* groups, where species are recognized primarily upon marked and consistent difference in ascospore patterns.

Aside from morphological similarities, the generally meagre growth of *A. citrisporus* and *A. ornatus* on synthetic media containing sucrose as a carbon source serves additionally to indicate their close relationship. A like response has been reported for *A. panamensis* (Raper and Thom, 1944). There, as here, the paucity of fungus growth is presumed to stem from an invertase deficiency. This limiting deficiency can be alleviated in each case by substituting glucose for sucrose. However, in the case of *A. ornatus* and *A. citrisporus* a further restrictive condition is imposed, and glucose should be supplied in an acid environment to attain a maximal response.

The discovery of an ascosporic stage in a species of *Aspergillus* assigned to a group wherein sclerotia have long been known, coupled with the fact that the perithecia themselves are at first parenchymatous and structurally resemble sclerotia, naturally raises the question, "What other species now regarded as sclerotium-producers may contain ascosporic strains?" Following a precedent established by Charles Thom, we have for years examined the sclerotia in newly isolated strains of *A. wentii*, *A. flavus*, and many other sclerotium-producers in the hope of finding an ascosporic form. In *Penicillium* the line separating

sclerotial and perithecial forms is thin indeed. Our search among the Aspergilli, like Thom's before us, has been in vain. Extension of our search for additional forms, and the introduction of new procedures for isolating them, may in time justify our optimistic hope. The possibility should not be dismissed lightly.

SUMMARY

Two additional species of *Aspergillus* are recognized as ascosporic, namely, *Aspergillus citrisporus* von Höhnel emend. and *A. ornatus* n. sp. Both species grow luxuriantly upon malt extract agar and other nutrient-rich natural substrata. Neither species grows well upon Czapek's solution agar with sucrose as a carbon source, but each grows satisfactorily when glucose is substituted for sucrose and the medium is rendered mildly acidic. Based upon cultural characteristics and upon details of morphology in the conidial phase, these species seem to belong near to or within the *A. tamarii* group. The perithecia in both species are strikingly similar and are markedly different from fertile structures previously reported in the genus *Aspergillus*. They are parenchymatous at first, become purplish at maturity, and superficially resemble the young sclerotia of various Aspergilli. These newly recognized ascosporic species cannot be placed in the *A. glaucus, A. nidulans,* or *A. fumigatus* groups, long established as ascigerous sections of the genus *Aspergillus*. It becomes necessary, therefore, to recognize a fourth group of species as potentially ascosporic. For the present it seems that such can best be aligned with the *A. tamarii* group.

ACKNOWLEDGMENTS

The writers wish to express their indebtedness to Professor Myron P. Backus, University of Wisconsin, for first recognizing and calling to our attention the unusual characteristics of *A. ornatus;* to the late Professor W. Lawrence White, Farlow Herbarium, for making Professor Thaxter's collections available to us; to Dr. Johanna Westerdijk, Centraalbureau voor Schimmelcultures, for returning to us a living culture of the strain of *A. citrisporus* which had been sent to her by Thom and Church, and

which was subsequently lost from our Collection; to Dr. Charles Thom, U. S. Department of Agriculture (retired), for personal consultation and access to cultural notes; to Edith K. Cash, Division of Mycology and Disease Survey, USDA, for preparing the Latin diagnosis of *A. ornatus;* and to Messrs. George E. N. Nelson and Roland Haines of the Northern Regional Laboratory for making sugar determinations and preparing illustrative material, respectively.

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A CLEISTOTHECIUM-LIKE STRUCTURE FOUND IN HORMODENDRUM PEDROSOI

H. Karrer and N. F. Conant

(with 1 figure)

One of the etiologic agents of chromoblastomycosis, *Hormodendrum Pedrosoi,* is known to reproduce in culture by at least three types of imperfect sporulation (9). Among the many strains which have been isolated, the lack of or the predominance of one or another of these types of sporulation also is known to occur (3). Such polymorphism exhibited by this fungus has made its exact taxonomic position a controversial subject for many years with the result that it has been placed in the following genera: *Acrotheca* (Fonseca and Leão, 1923); *Trichosporium* (Ota, 1928); *Gomphinaria* (Dodge, 1935); *Botrytiodes* (Moore and Almeida, 1936); *Phialoconidiophora* (Moore and Almeida, 1936); *Hormodendroides* (Moore and Almeida, 1936); *Fonsecaea* (Negroni, 1936); *Phialophora* (Emmons, 1944).

It has been suggested that this taxonomic problem can be resolved only when the perfect stage of *H. Pedrosoi* becomes known (4). Recently an advance has been made in this direction with a report of structures resembling immature cleistothecia in one strain of *Phialophora* (1). A similar structure also found in one strain of *H. Pedrosoi* will be the subject of this report.

MATERIALS AND METHODS

Sabouraud's glucose agar (4% Difco glucose, 1% Difco neopeptone, 2% agar), corn-meal agar (Quaker Oats Co., yellow enriched corn meal) as prepared by Benham (2) and corn-meal agar fortified with 1% Difco yeast extract were used in this study. The fungus was grown on the above media in Petri dishes, in tubed slants and on slide cultures as described by Riddell (11).

One particular strain of *H. Pedrosoi,* used in this laboratory for teaching purposes for a number of years, was found to produce cleistothecia-like bodies. Its original number has been lost, however, so that it cannot be identified as to original source. Numerous other strains of *H. Pedrosoi* were grown either separately or in combination with the above strain for the purpose of testing heterothalism and/or cross fertility.

RESULTS

The *Hormodendrum, Acrotheca* and *Phialophora*-type of sporulations were produced on all of the media by this strain of *H. Pedrosoi* (FIG. 1, e, f, g). Cleistothecia-like bodies reached their greatest number, largest dimension and more perfect development, however, on Sabouraud's glucose agar slide cultures at room temperature and could be examined microscopically from permanent stained preparations of such cultures (FIG. 1, a). They were observed to develop within two weeks as lateral outgrowths from the mycelium. They were greenish in color at first but became progressively darker with age. From small multicelled structures arising laterally from the mycelium and resembling multicellular chlamydospores (FIG. 1, d), they developed into large round, membranous to carbonaceous, cleistothecia-like bodies, 40 μ in size, with a parenchymic wall of one layer of polygonal cells, from the base of which arose many septate setae of varying length (FIG. 1, b, c).

Such bodies could also be observed on slide cultures of corn-meal 1% yeast-extract agar but they were less well developed, being smaller, more irregularly shaped and often lacking setae. On plain corn-meal agar slide cultures they were nearly always absent.

In Petri dish cultures the bodies were seen only occasionally when Sabouraud's glucose agar was used, but not at all on plates of corn-meal yeast-extract agar. They were not found on any of the above media in agar slant cultures.

Several attempts to demonstrate asci and ascospores within the cleistothecia-like structures were unsuccessful. Fully developed bodies were crushed in lactophenol-cotton blue only to yield

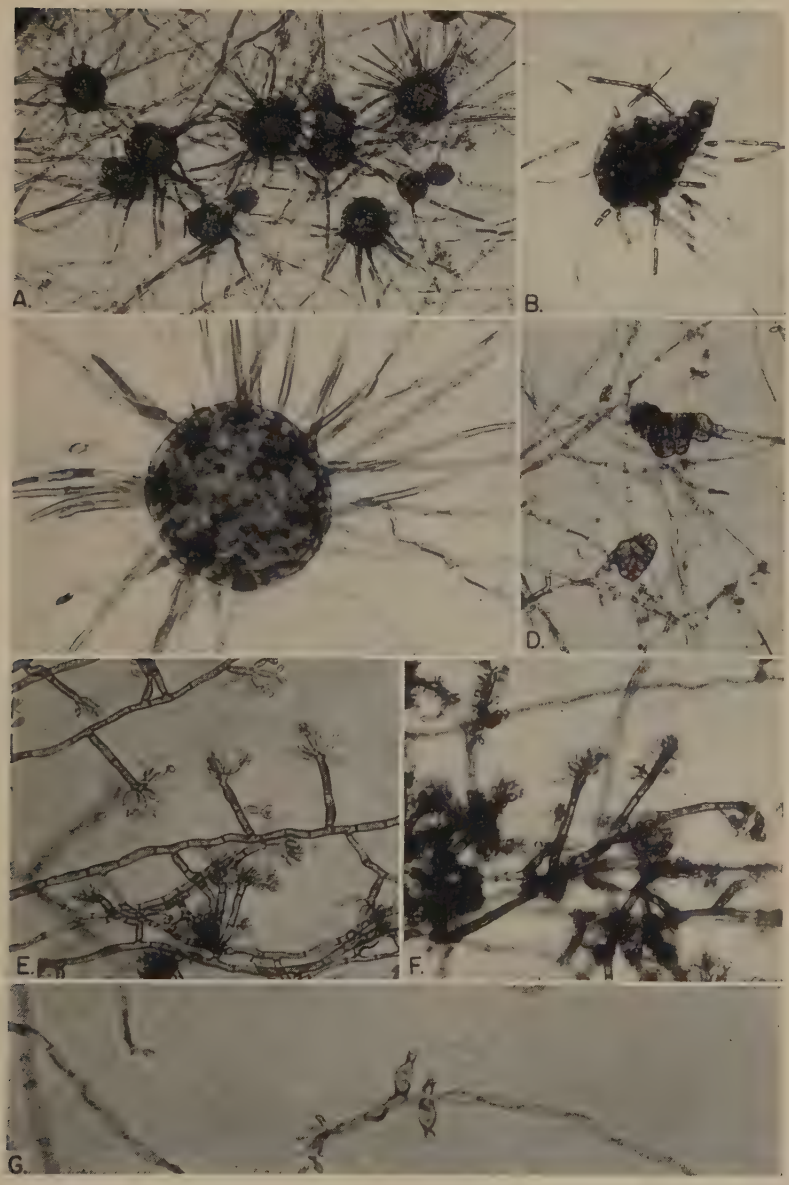

FIG. 1. *Hormodendrum Pedrosoi.* All photomicrographs taken from permanent preparations of Sabouraud's glucose agar slide cultures. A. Cluster of cleistothecium-like structures, × 254. B. Single beaked structure, × 350. C. Typical cleistothecium-like structure with setae, × 720. D. Small multicelled chlamydospore (sclerotium-like) bodies, × 350. E. Typical *Hormodendrum*-type sporulation, × 650. F. Typical *Acrotheca*-type sporulation, × 650. G. Typical *Phialophora*-type sporulation, × 870.

fragments of deeply stained parenchymous cells. Although such fragments superficially resembled asci with deeply stained internal structures, careful examination revealed their true nature.

Numerous other strains of *H. Pedrosoi* failed to develop cleistothecia-like structures when grown on the same media and under the conditions described above. An attempt to demonstrate heterothalism and/or cross fertility among these strains was not successful. To date, no strain or combination of strains grown in the neighborhood of the one forming cleistothecia-like structures, either in microcultures or on plates, has been able to stimulate the formation of mature ascus-bearing structures.

DISCUSSION

The morphology of the structure described for this one strain of *H. Pedrosoi* resembles exactly that found by Ajello (1) in one strain of *Phialophora verrucosa*. It is strongly suggestive of an immature ascocarp. Smaller, younger bodies which may be called chlamydospore-like, or sclerotia, have been observed previously by Carrión (5) and Carrión and Silva (7). The lack of asci may be due to the absence, under the conditions studied, of physical or chemical factors necessary for maturity or to the absence of proper strains to complete fertilization by one means or another.

After the discovery of a *Phialophora* type of sporulation in strains of *H. Pedrosoi* (6), it was suggested that the phialid-borne conidia might serve as spermatia (8, 10). In previous investigations, and in the present studies, however, attempts to cross-fertilize strains of *H. edrosoi* have not been successful.

The report of similar cleistothecia-like structures in strains of two fungi which cause chromoblastomycosis draws attention to the possibility that this group of fungi might ultimately be classified in a single genus of the Ascomycetes. This does not imply, however, that the classification of the imperfect stages, *Phialophora* and *Hormodendrum*, will be affected. In the first place, the imperfect generic name will carry over as, for example, *Aspergillus* for the imperfect stage of *Eurotium*. In the second place, the ultimate perfect stages of these fungi may be found to represent different species in the same genus each with its own, *Phialophora* or *Hormodendrum*, imperfect stage. An example of

such a situation recently has been described by Whitehead and Dickson (12) in which *Pyrenophora alternaria* was shown to have an *Alternaria* imperfect conidial stage and *P. secalis* to have a *Helminthosporium* imperfect conidial stage. It may still be necessary, therefore, for taxonomists to resolve the difficulties inherent in the taxonomy of the imperfect fungi causing chromoblastomycosis by means other than the discovery of their perfect stage.

SUMMARY

A new structure found in one strain of *Hormodendrum Pedrosoi* is described. The possibility of its representing an immature ascocarp is discussed.

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COMPARATIVE STUDIES OF FOUR RHIZOCTONIAS OCCURRING IN LOUISIANA

BEATRICE EXNER

(WITH 9 FIGURES)

Among the species of *Rhizoctonia* which have been reported in Louisiana there are four of particular interest from a pathological standpoint. These are responsible for diseases of considerable importance on certain crops. They are the ones which have been reported in the literature as *Corticium microsclerotia* Weber (*Rhizoctonia microsclerotia* Matz), the cause of web-blight of beans and other plants, *Corticium sasakii* Matsumoto, the cause of the banded scleroital disease of sugarcane, rice and certain grasses, the ubiquitous *Corticium solani* (Prill. and Del.) Bourd. and Galz. (*Rhizoctonia solani* Kuhn), the cause of root and stem diseases of many plants, and an undescribed *Corticium* which has been reported in Louisiana (16) as causing a leaf blight of figs.

The relationship and taxonomy of these four Rhizoctonias has been a problem among plant pathologists. Before the change in 1943 to *Pellicularia* by Rogers (13), *Corticium* had been accepted as the name of the genus to which the four fungi belonged, and each was considered a separate species by the workers who described the perfect stages. However, similarities observed among the basidiomycetous stages and certain cultural characters of single basidiospore isolates as reviewed or reported in this paper have thrown doubt on the validity of these species.

In preliminary studies (14, 1), it was found that a rather large number of cultural types occurred among single basidiospore isolates of these fungi. In 1921 Muller (11) found that basidiospore cultures of the fungus known as *Rhizoctonia solani* differed in certain characters and suggested that segregation of types probably occurred in the formation of basidiospores. It was felt that

further comparative studies of the cultural characters of tissue isolates, the basidiomycetous stages, and variations among single basidiospore cultures would reveal something of their relationship. Also, the single basidiospore cultures offered a means of studying the possible role that the basidiomycetous stages play in the origin of physiological races which have been reported by various investigators for *Rhizoctonia solani* (e.g., **15**, **5**) and *Corticium sasakii* (**7**).

In order that these fungi may be referred to separately without synonyms, the names used in the first part of this paper up to the discussion will be *Corticium solani, Corticium microsclerotia, Corticium sasakii,* and *Corticium* sp.

MATERIALS AND METHODS

The diseases caused by the four fungi can usually be distinguished by the symptoms produced on the various hosts which they attack. This was determined by isolations from the diseased hosts and the identification of the fungus found associated with the disease. Small pieces of diseased tissue were placed on water agar on which the pathogen soon outgrew all other organisms, and pure cultures were obtained from the leading edges of the mycelia. For identification the cultures were compared with representative cultures obtained from stock cultures at Louisiana Agricultural Experiment Station. Among the stock cultures was a culture of *Corticium sasakii* Matsumoto which had originally come from Matsumoto, a culture of *C. miscrosclerotia* Weber which had been obtained from Weber, and cultures of the fig blight organism obtained by Tims and Mills, who first reported the disease (**16**).

Descriptions of typical tissue isolates were made from cultures grown on potato dextrose agar (250 gms Irish potatoes, 20 gms dextrose, 15 gms agar, sufficient water to make 1000 cc) for two weeks at 28° C. Selected typical cultures were later grown on Czapek's agar under the same conditions to compare formation of sclerotia on this synthetic medium.

In periods of heavy rainfall and high humidity in June, July, and August, the basidiomycetous stages of these fungi have been

found in abundance on various hosts in Louisiana. Frequently extensive hymenia were found on plants which were not being parasitized. The fungi were merely growing over the surface and forming the basidial stage. Basidial mats from 14 hosts were examined and identified by the cultures arising from basidiospores or by cultures which came from plating out pieces of the basidial mats. Length and width measurements were made on 3285 basidiospores in mounts made from 62 collections. Basidia with fully extended sterigmata were rarely seen; however, at least ten from each of the four fungi were found and measured in length and width.

Several hundred single basidiospore cultures were isolated for comparative studies of the cultural characters and pathogenicity to determine the extent of variation within each group. These cultures were obtained by isolating germinating spores scattered over the surface of potato dextrose agar of about 1 mm depth in Petri dishes. Small strips of basidial mats were made to adhere to wet filter paper in the covers of sterile dishes containing the agar. The covers were moved around periodically to distribute the spores over the surface of the agar. After the spores had begun to germinate, isolated spores were marked, the agar was cut with a "biscut cutter," 1.5 mm in diameter, after the manner described by Keitt (4), and the pieces were transferred to sterile dishes, where they were examined to be certain that only one spore occurred on each piece. The single spores were then transferred to tubes of agar.

The cultures used in comparisons were grown under the same conditions as nearly as possible. They were first compared in tubes of potato dextrose agar, and the various strains found among the isolates were then transferred to plates of potato dextrose agar. After 48 hours growth, three uniform pieces of inoculum were cut with a 5 mm cork borer and transferred to triplicate plates, each containing 25 cc of potato dextrose agar. The agar used in each experiment was from a single batch.

The rate of growth was determined by measuring the diameters of the mycelia incubated at 28° C at the 24th and 48th hour. The difference between the two measurements was regarded as the increase in diameter per 24-hour period.

After two weeks growth at this same temperature the cultures were examined for other cultural differences—size, shape, number, and arrangement of sclerotia; color and topography of the mycelium; and ability of the cultures to darken the medium. The extent to which the cultures could be classified on the basis of descriptions of typical tissue isolates was determined and tabulated.

To determine differences in pathogenicity, bush snap beans were grown as the test host in sterilized soil artifically infested with a number of the isolates. The inoculum was prepared by growing the isolates for a week on a sterile oat-wheat mixture (four parts oats and one part wheat to which an equivalent weight of water was added). Two pots were used for each isolate. Twenty seeds to the pot were planted in the infected soil and watered from day to day. After 26 days the plants were removed from the pots, and the soil was washed away from the lower stems and roots so that the extent of injury could be determined. To measure the pathogenicity, an arbitrary scale of infection similar to the one described by Person (12) was used. Each plant was examined to determine the severity of lesions formed and then placed in one of the four following infection classes:

0 = no infection
1 = small lesions on hypocotyl and roots
2 = large lesions on hypocotyl and roots
3 = dead or dying plants

The index of pathogenicity was determined for each isolate by multiplying the number of plants in each group by the number assigned to that infection class and dividing the sum of the products by the total number of plants. A pathogenicity index of 1 would be moderately pathogenic, and of 2 or over would be severely pathogenic.

Ten cultures from a basidial mat of *Corticium solani* were tested as to damping off, using cabbage, tomato, and egg plant as test hosts. Three pots of sterilized soil were inoculated with each culture for each host, and 50 seeds were planted in each pot. The counts on the number of seedlings damped off were taken periodically for 18 days.

FIGS. 1–4.

SYMPTOMS OF THE DISEASES

Root and stem rots of various hosts (FIG. 1). This is the common group of diseases caused by the fungus known as *Rhizoctonia solani*. The symptoms are usually a rotting of the roots and cankers on the stems near the soil line. If the plants are very young, damping off may occur.

Web-blight (FIG. 2). This disease is characterized by a necrosis of the leaves and stems of various plants. The primary diognostic character is the occurrence of numerous small brown sclerotia from 0.2 to 0.5 mm in diameter, which look somewhat like grains of sand scattered over the surface of the host. Blighted leaves cling to other leaves or to stems, and when they are pulled apart it is seen that they are held together by cobweb-like threads. This disease was first reported in 1916 from Florida by Matz (8, 9) who named the causal organism *Rhizoctonia microsclerotia*. In 1940 Weber (17, 18) found basidial mats on bush snap beans affected with web-blight and named the organism *Corticium microsclerotia*.

This disease is known to occur on a large number of plants, but it has been found most frequently in Louisiana on three hosts—snap bean (*Phaseolus vulgaris*), lima bean (*Phaseolus lunatus*), and fig (*Ficus carica*),

Banded sclerotial disease (Fig. 3). This disease occurs most frequently on members of the family Gramineae, although it has been found on snap bean (*Phaseolus vulgaris*), lima bean (*Phaseolus lunatus*), *Dichondra repens,* peanut (*Arachis hypogea*), Alyce clover (*Alysicarpus vaginalis*), and *Commelina* species. The type of injury is distinct in that large, irregular, bleached spots occur in the leaves and sheaths. Sometimes a banded effect is produced on grass blades when the spots are numerous. Globoid sclerotia, 2–5 mm in diameter, are often produced on the diseased plants. The roots apparently are not affected, and the stems of

FIGS. 1–4. Disease symptoms produced by four Rhizoctonias: 1. Root and stem rot on young potato, caused by *Corticium solani*. 2. Web-blight on Celeste fig, caused by *C. microsclerotia*. 3. Banded sclerotial disease of grasses on *Eleusine indica,* caused by *C. sasakii*. 4. Leaf blight on Celeste fig, caused by *C.* sp.

diseased plants are only occasionally injured. The fungus causing this disease is evidently the same as the one called *Corticium sasakii* by Matsumoto (**6**) in 1934.

A leaf blight of figs (FIG. 4). This disease is similar in appearance to web-blight of figs caused by *Corticium microsclerotia* Weber and thread blight caused by *Pellicularia koleroga* Cooke. However, it can be distinguished from web-blight by the fact that no microsclerotia are formed on the host and from thread blight in that no mycelial threads and no flat, crusty sclerotia, characteristic of the thread blight fungus, are produced. This disease is caused by an undetermined *Corticium* and was first reported by Tims and Mills (**16**) in 1938.

One of the four fungi listed above, only *Corticium solani* has been isolated in nature from roots or lesions on basal stems. This indicated that in nature the other three fungi were confined to aerial parts of hosts attacked.

CULTURAL CHARACTERS OF THE FOUR RHIZOCTONIAS

When tissue isolates were made from the diseased material of the four diseases, a rather distinctive cultural form was found to be associated with each disease, but microscopic examination of the mycelia of the four forms revealed no characteristic by which one could be distinguished from the others. After two weeks growth on potato dextrose agar and Czapek's agar, they all possessed to a varying extent the short-celled, stout hypha, called sclerotial cells, which attained a diameter of 10–18 μ and had formed characteristic sclerotia in cultures of *Corticium microsclerotia, C. sasakii,* and the undetermined *Corticium.*

Although several tissue isolates were found to be almost identical in cultural characters with each of the representative cultures, some variation in cultural characters was observed among cultures in each group. However, the differences were so slight that classification was not difficult. The variations observed among cultures of *Corticium solani* were similar to those reported by Person (**12**), Houston (**2**), Kotila (**5**), and other workers, and the variations observed among cultures of *C. sasakii* were similar to those reported by Matsumoto, Yamamoto and Hirane (**7**).

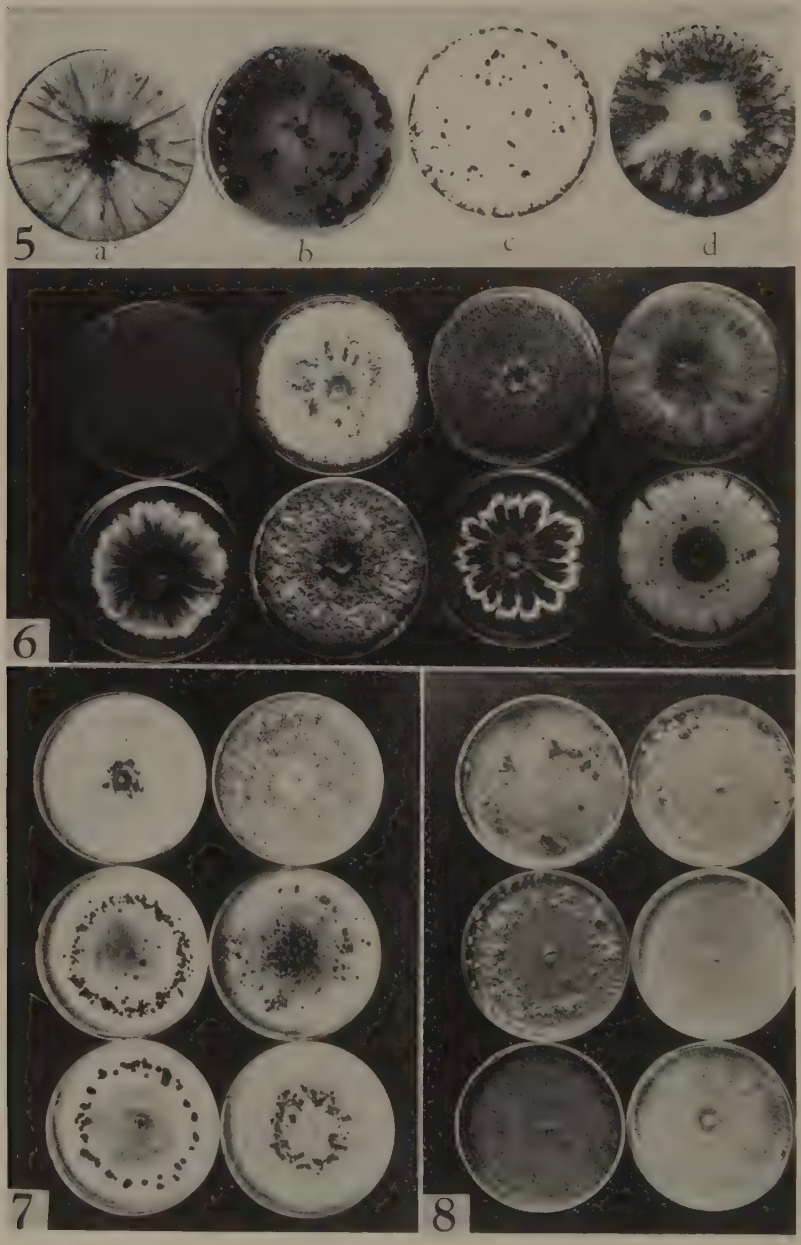
All cultures made from diseased plants covered with microsclerotia could easily be identified as *C. microsclerotia* and all cultures made from severely blighted fig leaves on branches devoid of sclerotia could easily be recognized as the undetermined *Corticium*. Formation of sclerotia by *C. solani, C. microsclerotia* and *C. sasakii* was practically the same on Czapek's agar as on potato dextrose agar. The undetermined *Corticium* produced a greater number of separate sclerotia which could be easily measured on Czapek's agar. FIG. 5 shows the four fungi grown for two weeks on potato dextrose agar.

The following descriptons were made from tissue isolates:

Corticium solani. The cultures in this category were variable in the production of sclerotial bodies, but were easily distinguished from the other three Rhizoctonias, which produced characteristic sclerotia. Some of the cultures produced small, irregular sclerotia scattered over the surface of the agar; some produced flattened coalescent sclerotia, usually in the center of the plate; and others produced radiating lines or thin patches of sclerotial cells on the surface of the agar.

Corticium microsclerotia Weber. Although the cultures in this group produced numerous, very small, grayish-brown sclerotia on the host, on media they produced few but very large sclerotia, which were tawny in color when young and deep brown at maturity. The sclerotia varied in size from 3–7 mm in width, from 4–11 mm in length, and 2–3 mm in depth. The average from 2 representative cultures was 4.4 mm in width, by 6.1 mm in length, by 2.6 mm in depth. The largest sclerotia, which were slightly irregular in shape, were usually the result of coalescence of two to four smaller sclerotia.

The fungus causing web-blight in Louisiana was evidently the same as the one described in 1939 by Weber (**17, 18**) in Florida, but a different strain from the one described by Matz (**10**) in 1921. The one which Matz called *Rhizoctonia microsclerotia* produced small sclerotia on both host and agar. Weber's *Corticium microsclerotia* produced small sclerotia on the host and large sclerotia on agar and therefore fitted more closely Matz's description of a strain which he called *R. dimorpha* (**10**).



FIGS. 5–8.

Corticium sasakii Matsumoto. The cultures in this group were characterized by the production of sclerotia on the agar which were rather definite in shape and size. They appeared spherical to ovoid from the top, but were slightly flattened underneath. They varied in size from 1–5 mm in width, from 1–6 mm in length, and from 1–2 mm in depth. The average for the sclerotia measured from nine isolates was 2.0 mm in width, by 2.6 mm in length, by 1.6 mm in depth. The sclerotia were first light colored and became deep brown, almost black, as they became older.

The undetermined Corticium. These cultures produced a large number of flattened, irregular sclerotia which coalesced to form rough, crusty aggregations over a large part of the surface of the agar and on the sides of the petri dish, when grown on potato dextrose agar. The sclerotial aggregations were grayish-brown to deep brown in color. On Czapek's agar many separate sclerotia were formed. They were round to elongate, ranging in size from 0.5–7 mm in diameter and 0.5–4 mm in depth, deep brown underneath with a lighter colored cottony top.

THE BASIDIOMYCETOUS STAGES

No distinguishing character was found by which the basidial mats of the four fungi could be separated. They appeared as thin, powdery, white layers over the surfaces of stems and leaves and became beige as they grew older.

The hymenial layer in each case was made up of a loose weft of articulate hyphae from which the basidia arose in small, imperfect cymes. The stalk cells apparently formed branch hyphae from which new basidia were developed.

The basidia were similar and agreed with descriptions given by various authors. They were barrel shaped or clavate, bearing four sterigmata (occasionally two) which arose as blunt knobs and later became horn-shaped.

FIGS. 5–8. 5. Typical tissue isolates of four Rhizoctonias on potato dextrose agar: a. *Corticium solani;* b. *C. microsclerotia;* c. *C. sasakii;* d. *C.* sp. 6–8. Single basidiospore cultures showing variations in cultural characteristics of isolates from a single hymenium: 6. from *C. solani;* 7. from *C. sasakii;* 8. from *C. microsclerotia.*

The basidia and sterigmata were variable within a single collection and the ranges of the measurements were overlapping among the four fungi. The basidia of *Corticium microsclerotia* ranged from 6.8–13.6 μ in length, averaging 10.6; 5.4–9.4 μ in width, averaging 7.1 μ; and the sterigmata ranged from 6.8–10.2 μ in length, averaging 8.7 μ. The basidia of *Corticium sasakii* ranged from 9.0–14.0 μ in length, averaging 12.4 μ; 7.0–10.0 μ in width, averaging 8.3 μ; and the sterigmata ranged from 5.0–9.0 μ in length, averaging 7.7 μ. The basidia of *Corticium solani* ranged from 10.2–17.0 μ in length, averaging 14.0 μ; 7.0–10.0 μ in width, averaging 8.8 μ; and the sterigmata ranged from 13.0–16.0 μ in length, averaging 14.2 μ. The basidia of the undetermined *Corticium* on fig ranged from 9.8–14.7 μ in length, averaging 12.2 μ; 6.5–9.8 μ in width, averaging 8.3 μ; and the sterigmata ranged from 6.5–12.2 μ in length, averaging 8.5 μ.

TABLE I

MEASUREMENTS OF BASIDIOSPORES OF FOUR RHIZOCTONIAS (CORTICIUM SPP.) FROM BASIDIAL MATS FORMED IN NATURE

	No. basidial mats	No. hosts	No. spores measured	Average in μ		Range in μ	
				Width	Length	Width	Length
C. solani	9	3	825	5.31	8.93	4.99–5.63	8.55–9.20
C. microsclerotia	12	5	583	5.15	8.30	4.70–5.78	7.68–9.44
C. sasakii	21	10	763	5.20	8.46	4.67–5.92	7.36–9.49
C. sp.	20	1	1114	5.52	8.54	4.67–6.24	7.55–9.47
Totals	62	19	3285				

The basidiospores produced by the four Rhizoctonias were found to be indistinguishable from one another. They were ellipsoid or oblong-ellipsoid, smooth, thin-walled, flattened on the side toward the center of the group, and truncate-apiculate.

The results of measurements of the large number of basidiospores taken from the 62 collections are given in TABLE I. The average dimensions of 583 basidiospores from 12 collections of hymenia formed by *Corticium microsclerotia* were 5.15 × 8.3 μ, of 763 basidiospores from 21 collections of hymenia formed by *Corticium sasakii* were 5.2 × 8.46 μ, of 825 basidiospores from 9 col-

TABLE II

INCREASE IN DIAMETER IN MM FROM 24TH TO 48TH HR ON POTATO DEXTROSE AGAR AT 28° C OF 269 SINGLE BASIDIOSPORE CULTURES OF FOUR RHIZOCTONIAS (CORTICIUM SPP.)

Basidial mat	No. cultures giving increases in diameter of					Total cultures
	0–10 mm	11–20 mm	21–30 mm	31–40 mm	41–50 mm	
C. solani						
A	0	0	4	2	0	6
B	0	0	8	13	0	21
C	1	1	9	9	1	21
D	0	1	5	17	1	24
E	1	3	5	5	1	15
F	1	4	4	10	0	19
G	0	0	5	7	1	13
J	0	0	8	19	2	29
C. microsclerotia						
I	1	1	14	3	0	19
M	4	1	1	10	4	20
C. sasakii						
S	0	1	4	10	10	25
C. sp.						
O	16	17	3	3	0	39
F	4	11	3	0	0	18

lections of *Corticium solani* were 5.31 × 8.93 μ, and of 1114 basidiospores from 20 collections of the undetermined *Corticium* on fig were 5.52 × 8.54 μ.

VARIATION AMONG SINGLE BASIDIOSPORE CULTURES

Corticium solani. In a previous note by Exner and Chilton (1) it was reported that comparative cultural studies showed a large number of distinct types among the progenies from single basidiospore cultures from 9 basidial mats from various hosts. As many as 29 distinct cultural types were found among the cultures from one small section of a basidial mat. These strains showed wide differences in their rate of growth, also. Further isolations made

after that showed the same occurrence of cultural differences (TABLE III) and differences in rate of growth among cultures from a basidial mat (TABLE II). However, all the cultures could be identified as belonging to *Corticium solani*, as it is differentiated from the other three Rhizoctonias in these studies (TABLE III).

TABLE III

CULTURAL STRAINS SECURED FROM SINGLE BASIDIOSPORE ISOLATES OF FOUR RHIZOCTONIAS (CORTICIUM SPP.)

Basidial mat	Host plant	No. of cultures isolated	No. of cultures compared in plates	No. of cultural strains	No. of cultures identifiable as same as parental type
		C. solani			
A	Lima bean	7	6	6	6
B	Lima bean	24	21	21	21
C	Lima bean	52	21	21	21
D	Lima bean	83	24	24	24
E	Alligator weed	60	15	15	15
F	Alligator weed	30	19	19	19
G	Irish potato	57	13	13	13
J	Irish potato	75	29	29	29
Q	Alligator weed	27	7	7	7
W	Alligator weed	6	5	5	5
Z	Alligator weed	16	8	8	8
X	Alligator weed	15	9	9	9
i	Alligator weed	38	35	20	20
j	Alligator weed	32	27	17	17
m	Alligator weed	20	12	12	12
	Totals	542	251	226	226
		C. microsclerotia			
I	Privet	49	21	21	10
M	Privet	26	21	21	10
	Totals	75	42	42	20
		C. sasakii			
s	Bermuda grass	51	25	20	18
		C. sp.			
o	Fig	39	39	25	12
f	Fig	18	18	11	6
	Totals	57	57	36	18

FIG. 6 shows cultures of different strains grown under similar conditions.

Corticium microsclerotia. Only about one-half of these cultures were easily classified as belonging to this group (TABLE III). As many as 21 distinct cultural types were found among the 26 cultures isolated from one basidial mat. Some were similar to those observed among cultures of *C. solani.* The rate of growth varied considerably. The average increase in diameter ranged from a mere trace to 50 mm per 24-hour period (TABLE II). The color of the mycelium ranged from cream to deep brown. Sclerotial formation ranged from patches or tufts of sclerotial cells, about 0.5 mm across, to thick sclerotial bodies more than 10 mm across. In some cultures the large sclerotia were pocked where droplets of exudate had formed; in others they were covered with cottony mycelium. The topography of the cultures ranged from a felty appearance through varying degrees of tuftiness to a dense wooly growth (FIG. 8).

Corticium sasakii. Among the single basidiospore cultures from *Corticium sasakii* about one-fourth were atypical cultures (TABLE III). The rate of growth varied from an increase in diameter of 12.4 mm to 50.5 mm per 24-hour period (TABLE II). The color of the mycelium ranged from white to brown, but in most cases it was still light at the end of two weeks. Some of the cultures showed no sclerotial formation except dark patches on the surface of the agar which showed through when the plate was held to the light. Other cultures produced sclerotia of varying sizes and number. The most common form was an almost spherical, occasionally elongated, deep brown, pocked body averaging between 2 and 3 mm across. However, in one culture observed, the sclerotia had attained a diameter of over 10 mm (FIG. 7).

Corticium species. The single basidiospore cultures from the undetermined Corticium gave the greatest proportion of atypical cultures. Only about one-third were easily recognizable as belonging to the *Rhizoctonia* from which they came (TABLE III). On the average, the rate of growth was considerably less than that observed among the cultures in the other three groups. The

range in increase in diameter was from 2 to 39 mm per 24 hour period (TABLE II). The color of the mycelium ranged from cream to deep brown. Sclerotial formation ranged from a thin stroma of sclerotial cells through the flat, crusty, coalescing sclerotia typical of this group to separate, large, raised sclerotia, about 8 mm across. The flat, crusty sclerotia were usually located around the edges of the plates, whereas sclerotia of the other forms were either in rings or scattered over the surface of the agar. The mycelium in most cases was felty in apperance, but in a few cases it produced extensive aerial growth (FIG. 9).

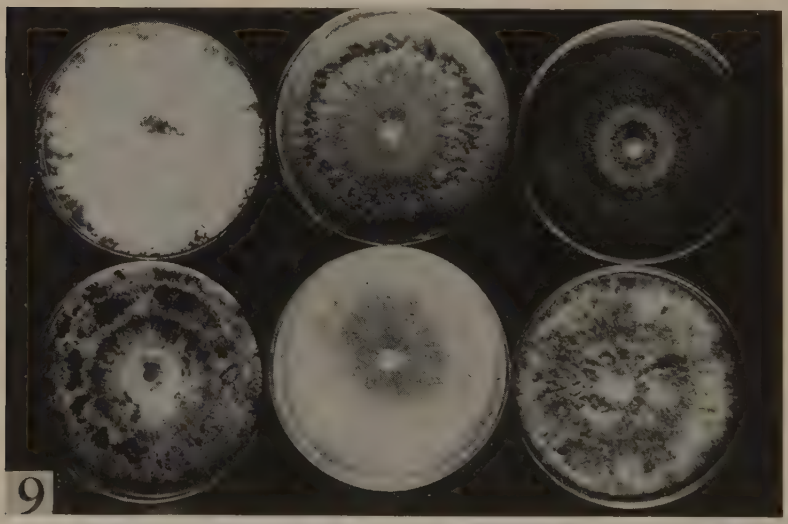


FIG. 9. Single basidiospore cultures of *Corticium* sp. showing variation in cultural characteristics among isolates from a single hymenium.

VARIATIONS IN PATHOGENICITY AMONG SINGLE BASIDIOSPORE CULTURES

The results of the pathogenicity tests (TABLE IV) indicated extensive variation in the ability of the single basidiospore cultures to damage the tissues of bush snap bean seedlings.

All four of the fungi produced lesions on the hypocotyl at the level of the soil and below. The only difference found in the lesions was in color. The cultures of *Corticium microsclerotia* and *C. sasakii* produced chocolate brown lesions, and the cultures

of *C. solani* and the undetermined *Corticium* produced reddish brown lesions. Among the cultures of *Corticium solani* pathogenicity ranged from slightly pathogenic to severely pathogenic. The cultures of *Corticium microsclerotia* showed a range of pathogenicity from non-pathogenic to slightly pathogenic. (Although

TABLE IV

PATHOGENICITY OF 113 SINGLE BASIDIOSPORE CULTURES OF FOUR RHIZOCTONIAS (CORTICIUM SPP.) TO BUSH SNAP BEAN IN STERILIZED SOIL IN GREENHOUSE

Basidial mat	Number cultures with pathogenicity index of						Total cultures
	0	0.1–.5	0.6–1.0	1.1–1.5	1.6–2.0	2.1–2.5	
C. solani							
A	0	1	1	0	1	0	4
B	0	1	4	1	1	0	7
C	0	2	1	4	1	2	10
D	0	1	2	3	2	1	9
E	0	3	1	0	2	2	8
J	0	6	3	5	0	0	14
C. microsclerotia							
I	3	10	0	0	0	0	13
C. sasakii							
W	0	1	5	1	1	1	9
C. sp.							
O	3	11	3	7	5	0	29
F	7	11	2	0	0	0	20

0 = non-pathogenic.
3 = plants dead or dying.

these cultures were the least pathogenic of the four Rhizoctonias on the roots and hypocotyls of bush snap bean seedlings, in fields the aerial parts of older bush snap bean plants may be totally killed by web-blight.) The cultures of *Corticium sasakii* ranged in pathogenicity from slightly pathogenic to severely pathogenic. The cultures of the undetermined *Corticium* ranged in pathogenicity from non-pathogenic to moderately pathogenic.

In the damping off tests with ten cultures from a single mat of *Corticium solani,* the stands of cabbage, tomato, and eggplant varied extensively (TABLE V). An analysis of variance indicated significant differences among the cultures.

TABLE V

STANDS OF CABBAGE, TOMATO AND EGGPLANT IN SOIL INFESTED WITH 10 BASIDIOSPORE CULTURES OF CORTICIUM SOLANI BURT

Culture	Percent stand in relation to check		
	Cabbage	Tomato	Eggplant
B–5	42.8	82.5	51.3
B–6	8.5	30.6	37.5
B–8	56.5	51.9	62.5
B–10	32.7	47.2	33.6
B–11	1.6	11.9	3.8
B–12	1.6	44.4	37.5
B–14	4.6	83.3	14.1
B–19	80.8	99.2	104.2
B–21	63.4	85.3	71.2
B–23	2.3	40.8	1.1

F value = 810.64. F value exceeds 1% point.

DISCUSSION

When basidiospore cultures of the four fungi were compared a large number were found which could not be identified by cultural characters or pathogenicity on bush snap bean. This was found to be the case even when the spores were taken from small sections of basidial mats. Many of the atypical cultures produced by *Corticium microsclerotia* and the undetermined *Corticium* on fig would have to be considered as *C. solani* since they possessed no cultural characters which would enable them to be distinguished from that group. Cultures from basidiospores produced by *C. sasakii* and *C. microsclerotia* intergraded so that some of them could not be assigned to either group. (Since some of the cultures of *C. microsclerotia* produced sclerotia on agar as small as those on the host, it seems highly probable that the fungus which Matz described as *R. microsclerotia* (9, 10) was a strain possessing the same cultural characters).

The results obtained in these studies indicate that the sexual stages of these fungi were responsible for a wide variety of strains,

many of which differed from the type considered to be representative of each form. This suggests that the fusion nuclei in the basidia are heterozygous and that strains arise as a result of segregation. The possibility exists that several strains could have produced basidiomycetous stages at the same time in the case of each of the basidial mats from which basidiospores were obtained. However, the large number of strains found among the progeny from a single mat, for example, the 29 distinct strains among 75 cultures from a mat of *Corticium solani* and the 21 distinct strains found among the 26 cultures from a mat of *C. microsclerotia,* has led to the interpretation that segregation is responsible.

The relationship of these four fungi seems to be very close. The basidial mats are practically indistinguishable, and the basidiospores cannot be distinguished by their size. The basidia and sterigmata are similar except for slight differences in measurements, the ranges of which are overlapping.

It is the opinion of Rogers (**13**) that the differences between *Corticium microsclerotia* Weber and *Corticium solani* (Prill. & Del.) Bourd. & Galz. fall within the range of a single species—*Pellicularia filamentosa* (Pat.) Rogers. Since these two fungi represent the extremes in average measurements of basidia and sterigmata among the four Rhizoctonias used in these studies, the other two, *Corticium sasakii* Matsumoto and the undetermined *Corticium,* would be placed in the same species.

As has been pointed out in the first part of this paper, the diseases caused by the four Rhizoctonias are reasonably distinct, and when isolates are obtained from diseased plants, a rather clear-cut cultural strain is found associated with each disease. The form and size of sclerotia formed when the cultures were grown under definite conditions was found to be the most practical way of separating them in the laboratory.

Therefore, it is proposed that the species, which according to Rogers (**13**) is entirely parasitic, be sub-divided for reference to the causal organisms of the various diseases.

It was thought best to call the four Rhizoctonias used in these studies forms (formae specialies), because they are separated on the basis of cultural characters and diseases produced, and not on

morphological differences of the perfect stages, as are subspecies and varieties. This is in conformity with Article 14 of the International Code of Botanical Nomenclature of 1952 (3). The following descriptions have been prepared for their identification:

PELLICULARIA FILAMENTOSA (Pat.) Rogers, Farlowia 1: 113. 1943.

1. Forma specialis **solani** (Kuhn) stat. nov.

Hypochnus solani Prill. & Del., Soc. Myc. Fr. Bull. **7**: 220, fig. *1991*. Muller, Biol. Reichsanstalt. Land- u. Forstwiss. Arb. **13**: 205–8, pl. *1, 2*. 1924.

Corticium vagum var. *solani* Burt ex Rolfs, Science N.S. **18**: 729. 1903. Missouri Bot. Gard. Ann. **13**: 295. 1926.

Corticium solani (Prill. & Del.) Bourd. & Galz., Soc. Myc. Fr. Bull. **27**: 248. 1911.

Corticium vagum sensu Burt, Mo. Bot. Gard. Ann. **5**: 128, fig. 3a. 1918.

Corticium vagum subsp. *solani* (Prill. & Del.) Bourd. & Galz., Hym. Fr. 242. 1928.

Botryobasidium solani (Prill. & Del.) Donk, Nederl. Myc. Ver. Med. **18–20**: 117. 1931; Rogers, Univ. Iowa St. Nat. Hist. **17**: 18. 1935.

The causal organism of basal stem and root rots of various plants.

Variable in the production of sclerotia in two weeks time on potato dextrose agar and Czapek's agar at 28° C. Some cultures produce numerous small (less than 2 mm), tawny to deep brown sclerotia which may coalesce to form flattened aggregations, and others produce only a layer of sclerotial cells over a large part of the agar or form a felty stroma.

2. Forma specialis **microsclerotia** (Matz) stat. nov.

Corticium microsclerotia Weber, Phytopath. **29**: 565, fig. *4, 7*. 1939. Mycologia **43**: 727. 1951.

The causal organism of web-blight of bean, fig, and various other plants. Numerous small, brown, superficial sclerotia, 0.2–0.5 mm in diameter on aerial parts of host.

Producing in two weeks time on potato dextrose agar or Czapek's agar at 28° C tawny to deep brown sclerotia, 3–7 mm in width, by 4–11 mm in length by 2–3 mm in depth.

3. Forma specialis **sasakii** (Shirai) stat. nov.

Hypochnus sasakii Shirai, Bot. Mag., Tokyo **20**: 319. 1906. *Corticium sasakii* (Shirai) Matsumoto, Trans. Sapporo Nat. Hist. Soc. **8**: 115–120. 1934.

The causal organism of a leaf and sheath spot (banded sclerotial disease) on grasses and a leaf blight on various dicotyledonous plants.

Producing in two weeks time on potato dextrose agar or Czapek's agar at 28° C deep brown sclerotia of definite shape—almost spherical (flattened where they come in contact with the agar and occasionally elongated), varying in size from 1–6 mm in diameter.

4. Forma specialis **timsii** forma nov.

Parasitus luem in foliis *Fici Caricae* efficiens. Nulla sclerotia in hospite gignuntur. Diebus quattuordecim in agaro Solani tuberosi dextrosato 28° C numerosa sclerotia complanata et brunnea efficit, quae ita coalescunt ut collationes asperae (Sclerotiorum) per magnam partem superficiei agari formentur. Diebus quattuordecim in medio synthetico (e formula Czapekii) cui grammata quindecim agari addita sunt) sclerotia efficit globosa vel elongata, atro-brunnea infra sed tegmina colore dilutiore et gossypio similia gerentia, magnitudine 0.5–7 mm in diametro ac altitudine 0.5–4 mm.

The causal organism of a leaf blight on fig. No sclerotia produced on host.

Producing in two weeks time on potato dextrose agar at 28° C numerous flattened, brown sclerotia which coalesce to form rough, sclerotial aggregations on a large part of the surface of the agar and sides of the Petri dish. Producing in two weeks time on Czapek's agar at 28° C sclerotia which are round to elongate, deep brown underneath with a lighter colored, cottony covering, ranging in size from 0.5–7mm in diameter and 0.5–4 mm in depth.

SUMMARY

A study was made of the four Rhizoctonias known as *Corticium solani, Corticium microsclerotia, Corticium sasakii,* and an undetermined *Corticium* species from fig. Each causes a rather characteristic disease. Tissue cultures from diseased material gave rather distinct cultural types for each disease. However, when basidiospores were isolated, and the cultures obtained from them were compared, a large number of types differing in appearance, rate of growth and pathogenicity were found. In many cases strains from basidiospores of one Rhizoctonia could not be distinguished from strains obtained from basidiospores of the other three Rhizoctonias.

The four Rhizoctonias could not be distinguished by average measurements of basidiospores or basidia.

The taxonomy of these fungi is discussed and four forms belonging to the species *Pellicularia filamentosa* are suggested in place of giving them specific rank.

ACKNOWLEDGMENTS

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DEVELOPMENT OF THE CARPOPHORE OF BOLETINUS PALUSTER

Arthur C. Gentile and Walter H. Snell

(with 1 figure)

The beautiful little *Boletinus paluster* (Pk.) Pk. is not common but when it does fruit, dozens to hundreds of carpophores are formed within a small compass. In 1941 Henry A. C. Jackson and the second author made an abundant collection near the railroad station at Vaucluse, Quebec, in a wooded area which deserves especial attention by those interested in the higher fungi because of the size of the woodland and the diversity of terrain and vegetation. This collection provided the material to add one more developmental study to the few made in the Boleti. The material was embedded, sectioned and stained by Miss Hilda Vilkomerson.

Space will be taken for no extended discussion because the general course of development of the carpophore can be ascertained from Fig. 1. When the primordia of the carpophores are 3–5 mm tall and the apical portion is 300–500 μ thick, the beginning of the pileus and the clothing layer are evident. Fig. 1, A, represents one of the larger conditions. When the pileus is only about 0.5 mm broad, it is well on its way to development, with a differentiated surface layer and a palisade layer of hyphal tips on the lower surface, and with the margin incurved and the marginal, hyphal weft approaching the stipe (Fig. 1, B).

When the pileus is 800 μ broad (Fig. 1, C), the hymenial palisade is more chromophilous, and at about 900 μ (Fig. 1, D) cystidia are evident, the marginal weft has made contact with the stipe, and the surface clothing appears much like that of the more mature stages.

The marginal weft may start to pull away from the stipe at the 1 mm stage (Fig. 1, F) or it may remain attached until the pileus is nearly 2 mm broad (Fig. 1, G), and even at the 4 mm stage

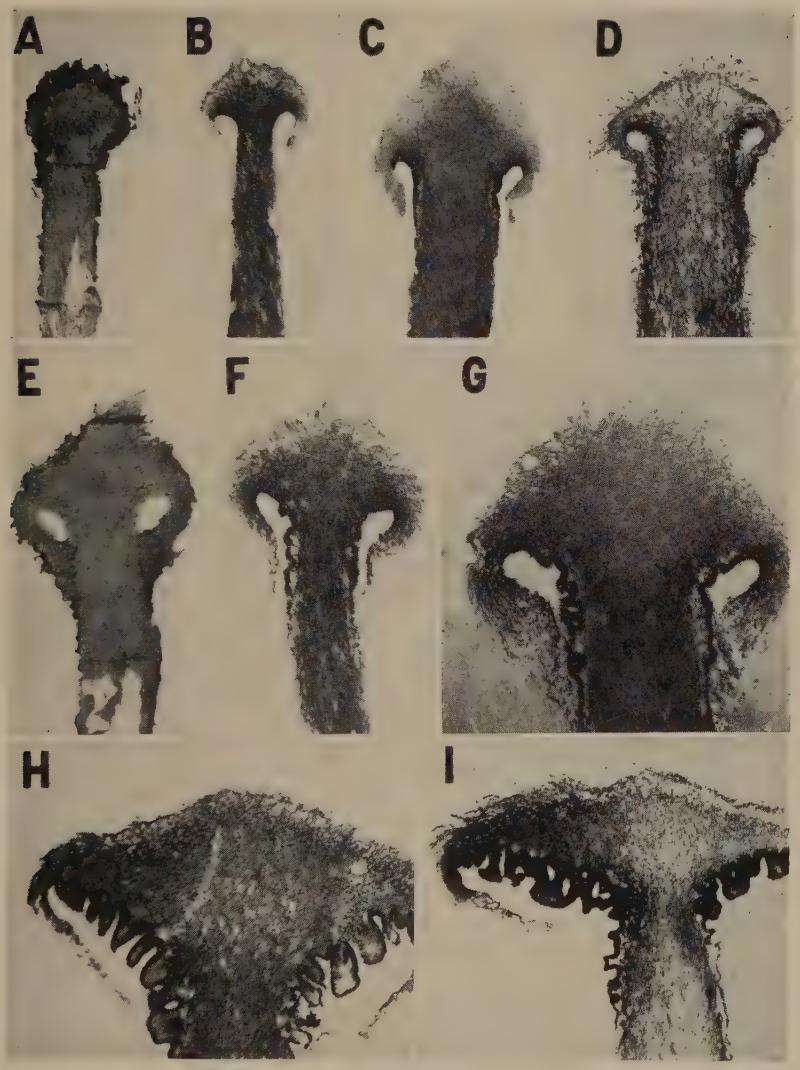

FIG. 1, A–I.

FIG. 1. Development of the carpophore of *Boletinus paluster*. A. Earliest stage, rather large, with knob-like pileus 550 μ broad, stipe slightly constricted at apex and surfaces of pileus and stipe covered with loose, projecting hyphae. B. Pileus 550 μ broad, showing definite pileus with incurved margin and marginal hyphae growing toward the stipe, palisade layer on the lower surface of pileus and a short way down the stipe, and surfaces of pileus and stipe of loose, hyphal tissue. C. Pileus 800 μ broad, with thick layer of

(FIG. 1, I) it has not retracted very far. In the mature carpophore, the veil remains appendiculate on the pileus and there is very little, if any, annulus on the stipe.

FIG. 1, G, H and I, show the irregular and lacunose development of a very boletinoid hymenophore.

The first mature basidia and basidiospores appear in small numbers at about the 1.5 to 2 mm stages of breadth of the pileus but are at all frequent only when the pileus is 2.5–3 mm broad, or between the stages represented in FIG. 1, H and I.

The type of development in *Boletinus paluster* is, therefore, pseudoangiocarpic.

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loose hyphae on the surface, more clearly defined palisade layer and further incurving of marginal hyphae. D. Pileus 880 μ broad, with pileic margin in contact with stipe completely enclosing the hymenial cavity, a definite hymenium of cystidia but no mature basidia, and looser clothing. E. Pileus 800 μ broad, showing complete and firm contact of the marginal tissue with the stipe, but details of all tissues lost because of poor focus. F. Pileus 1 mm broad, with marginal tissue slightly pulled away from the stipe and convoluted hymenium with abundant cystidia. G. Pileus expanded to 1.6 mm, with marginal tissues still in contact with the stipe and hymenophore lacunose as well as convoluted. H. Pileus 1.9 mm broad, almost completely expanded, with the marginal tissue free from the stipe but not far withdrawn, and tubes well started toward development. I. Pileus 4 mm broad and stipe about 1 mm thick with irregularity of tube-layer evident on pileus and upper stipe, and marginal tissue still much incurved. A–H about × 40; I about × 20.

CYTOLOGICAL OBSERVATIONS ON THE ZYGOTE OF MONOBLEPHARELLA[1]

FREDERICK K. SPARROW[2]

(WITH 1 FIGURE)

The Monoblepharidales, a small order of freshwater and terricolous phycomycetes, is unique in having a type of sexual reproduction involving a large non-flagellated egg and a disproportionately small, posteriorly, uniflagellate antherozoid. In the genus *Monoblepharis* Cornu (1872), species of which are typically found in cool, clear water, the egg completely engulfs the male gamete and the resultant zygote oozes out of the oogonium and becomes converted into a thick-walled oospore which remains attached to the orifice of the oogonium. In *Monoblepharella* Sparrow (1940), whose species have thus far been found only in tropical and subtropical soils, the flagellum of the male gamete persists after fertilization, the zygote emerges and swims away propelled by that flagellum. Such a unique type of behavior has been verified by Shanor (1942) and Springer (1945) in other species of the genus and has also been recently discovered by Johns (1952) in species of the well known genus *Gonapodya*.

In such a type of sexual reproduction as found in *Monoblepharella* one of the points of greatest interest is to determine just what nucleus presides over the activities of the flagellum during the motility of the zygote. Is there immediate nuclear fusion and does the diploid nucleus function in this respect, or is it one or other of the gamete nuclei?

Owing to the small size of the structures involved, sectioning of material did not seem feasible. Rather, methods similar to those employed by Kniep (1929) and others in studying the nu-

[1] Contribution No. 979, Botany Department, University of Michigan.

[2] Acknowledgment is made to the Executive Board of the Horace H. Rackham School of Graduate Studies for financial support given this project.

clear condition in the motile structures of *Allomyces* were employed. Cover slips from hanging drop cultures in which zygotes were swimming were removed from the Van Tieghen rings and the material killed, fixed, and strained on the cover slip.[3]

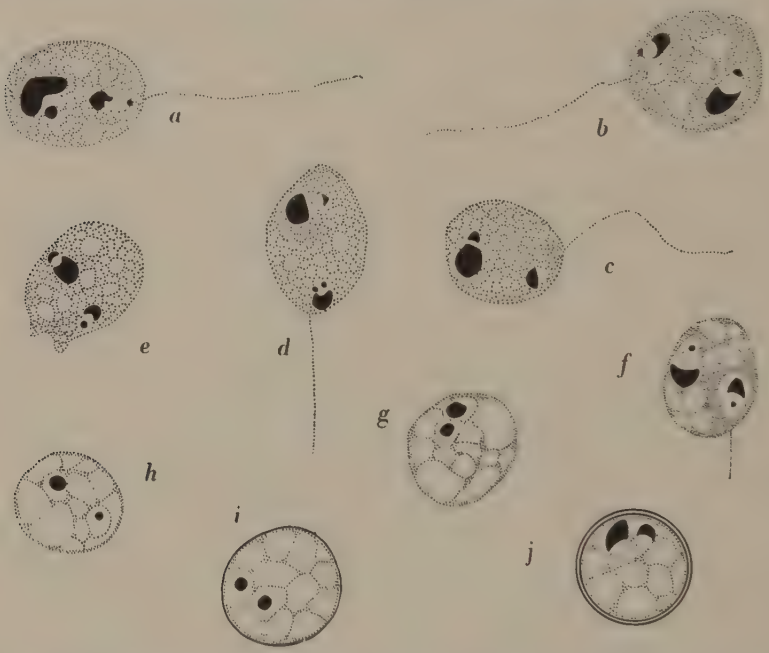

FIG. 1. *Monoblepharella taylori*. All Figs. × 1300. a–d, f. Killed, fixed and stained zygotes bearing flagella. In each instance the smaller nuclear apparatus of the male, with its nuclear cap lies near the flagellum, the larger apparatus of the female gamete more anterior. In a, f, the male nuclear apparatus and flagellum are in the position they probably assume during zygote motility. e. Zygote devoid of flagellum, beginning to assume amoeboid movement before rounding off and encysting. g, h. Rounded off zygotes prior to wall formation. i. Beginning of wall formation. j. Nearly mature oospore.

Owing to the small size of the nuclear apparatus (4.75 μ female gamete, 2.5 μ male gamete, including "nuclear cap") and the limitations inherent in the use of whole mounts, no attempts were

[3] Of the various standard killing and fixing fluids, Bouin's seemed to be most serviceable. Iron-alum hematoxylin stain, destained with picric acid in 70% alcohol gave a particularly sharp differentiation of the nuclear apparatus.

made to study the finer details of the nucleus itself. Indeed, for purposes of the study as outlined, this was not necessary. It will be recalled that a highly characteristic feature of the nucleus of all posteriorly uniflagellate motile bodies formed by Phycomycetes is the presence on the anterior periphery of a deep-staining "nuclear cap" of unknown function. This is ordinarily not regarded as being a part of the nucleus proper. At the opposite side is a deep-staining granule, the nucleole, intimately connected with the flagellum. Since nuclear cap size always bears a definite and constant relationship to the size of the motile cell which contains it, and since the sperm and egg are considerably different in size (sperm $5\,\mu \times 3\,\mu$ compared with $13\,\mu \times 10\,\mu$), attention may be directed primarily to the nuclear caps.

In FIG. 1, a–d, f, are shown stained zygotes with their flagella. Within the zygote are seen two nuclei with distinctly different sized nuclear caps. In each instance the larger of the two nuclear structures is near the anterior portion of the zygote and the smaller near the point of insertion of the flagellum. In FIG. 1, e is shown a zygote which has evidently lost its flagellum and is about to move amoeboidly before encysting. Here, too, the nuclear structures are distinct and different sized. It is evident, therefore, from these few examples of many similar ones which might be shown, that the nuclei of the two gametes remain distinct within the motile zygote and that the smaller, undoubtedly male gamete nucleus maintains its connection with the flagellum and governs its activities.

After the zygote comes to rest in the water and loses its flagellum, it rounds up and there is gradually secreted a somewhat thickened wall, which at maturity of the oospore is smooth and somewhat brownish. FIG. 1, g, h, indicate that during this encystment and oospore wall formation the nuclear caps, and probably the nuclei, remain distinct. In FIG. 1, j, a recently matured oospore is shown and here, too, the nuclear caps remain distinct.

From this brief study it is apparent that the nucleus of the male gamete rather than a fusion nucleus presides over the activities of its flagellum during motility of the zygote and that the nuclear caps, and probably also the nuclei, remain distinct in the early

stages, at least, of oospore formation and maturation. Fully mature oospores are difficult to stain *in toto*. From what can be determined, however, only a single nucleus is present. Nuclear behavior in the oospore appears to parallel that found in *Monoblepharis* by Lagerheim (**1900**) and Laibach (**1926**) where here, too, the nuceli lie side by side until an advanced stage of wall maturation, when they eventually fuse.

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THE GENUS BEAUVERIA, MORPHOLOGICAL AND TAXONOMICAL STUDIES OF SEVERAL SPECIES AND OF TWO STRAINS ISOLATED FROM WHARF-PILING BORERS

RHODA W. BENHAM[1] AND JOSE L. MIRANDA[2]

(WITH 5 FIGURES)

The genus *Beauveria* is of interest both historically and from the fact that it contains many well known insect parasites, including the agent of *"la muscardine"* or silkworm disease. This is one of the most commonly known species, because of its economic importance and because of the epoch-making studies of Pasteur. It has acquired recognition among bacteriologists and medical mycologists as it is usually cited in text books on these subjects as the first instance in which a disease in man or animal was proven to be due to a definite micro-organism. It was as early as 1835 that Bassi studied and described the disease and thus established precedents for future workers. To quote Gay, 1935, "The proven pioneer work of Bassi on the muscardine disease of silkworm to which we have already referred, established a standard for proving the etiological relationships of a micro-organism found in diseased tissues. He realized the specific significance of the particular fungus involved and transmitted the malady from diseased to healthy worms."

Bassi was not a mycologist and therefore sent the cultures to Balsamo-Crivelli for study, who placed the fungus in the genus

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Botrytis, naming it *Botrytis Bassiana* (Balsamo **1835**). Later studies by other authors indicated that the organism was similar to other insect parasites being isolated from various localities and that the characteristics were not those of a *Botrytis.* However, other species were described under this name. Delacroix (**1891**) described as *Botrytis tenella* a similar species obtained from cockchafer. Giard (**1891**) showed that this fungus had been described by Link in 1809 as *Sporotrichum densum.* Giard, however, noted that the fungus formed coremia and adopted the name *Isaria densa* (Link) Fr. Delacroix did not agree with Giard and retained the name *Botrytis tenella.*

Saccardo (**1886**) described a *Botrytis tenella* as a sub-species of *B. Bassiana* in Sylloge Fungorum **4**, p. 119. He did not, however, mention the characteristic conidial heads of the *Botrytis Bassiana* group and there seems to be some doubt as to whether the species is allied to *B. Bassiana.* Spegazzini (**1880**), in South America, described from dead carabid beetles another species as *Sporotrichum globuliferum.* This name has been applied, as pointed out by Dodge, in America to the fungus which parasitizes the chinch bug, *Blissus leucopterus.* Pettit (**1895**) studies this species and observed the purplish color mentioned below. Thus it will be seen that two generic names have been given to what appear to be similar insect parasites, *Botrytis* being the name more commonly used by European mycologists, and *Sporotrichum* that preferred by the Americans. The organisms in question do not fit in either group. Vuillemin (**1912**), realizing this, created the new genus *Beauveria* for these forms, and characterized the genus by the unique type of conidiophore, suggesting a cyme or sympodium. The sporogenous filament forms a prolongation of the phialide and the disposition of the spores gives a zigzag arrangement. *Botrytis Bassiana* then became *Beauveria Bassiana* (Balsamo) Vuill. Picard (**1914**) transferred Spegazzini's species to *Beauveria* as *Beauveria globulifera* (Speg.) Picard. Beauverie (**1914**) added a new species, *Beauveria effusa,* and published a critical study of the genus. He concludes that there are four species, which he lists as follows:

(1) *Beauveria Bassiana* (Balsamo) Vuillemin 1911
(2) *Beauveria effusa* (Beauverie) Vuillemin 1911
(3) *Beauveria densa* (Link) Picard 1914
(4) *Beauveria globulifera* (Spegazzini) Picard 1914

Beauverie states that these species are all very similar, the most startling differences being in the gross characteristics. They are all known to be insect parasites.

Petch (1924) also gives a critical survey of the group and reports on studies with strains from various sources. He concludes that the morphological differences between alleged species are slight and that from recorded experiments it does not seem probable that the parasitism of a given species is restricted to one species of insect or even one group of insects. Lefebvre (1921) had shown that both *B. Bassiana* and *B. globulifera* could attack the corn-borer *Pyrausta nubilatis*. The strain *B. Bassiana* appeared more infectious, however. He gives illustrations that are typical of the genus and agrees with others that the two forms are similar morphologically but that *B. Bassiana* produces a chalky growth and *B. globulifera* a flocculent growth.

In spite of the vast literature on these insect-parasites and the many excellent illustrations of their characteristic morphology we find a report by Limber (1940) on "A new form genus of the Moniliaceae." He found his fungus in dead roots of a *Yucca* plant of Cuban origin. The conidium-bearing filaments had a zigzag appearance and since it seemed similar to the rachis of a wheat spike, he created a new genus and named it *Tritirachium* and the type species *dependens*. This, he states, can readily be distinguished from other members of the Moniliaceae by the zigzag form of the fertile portion of the conidiophore. The only other reference he was able to find in the literature to this zigzag formation was that of Ferraris (1912), who described under the name *Sporotrichum flavicans,* a fungus which was found on the underside of cork placed in a flask of picric acid. Limber describes a second species under the name *Tritirachium album*. This he received from Dr. Charles Thom, who found it associated with a colony of *Penicillium intricatum* in material he had obtained from Dr. Oscar Richards, who had recovered it from a book in the basement of

the Marine Laboratory in Woods Hole. Limber's illustrations differ from the typical *Beauveria* picture in some respects but the zigzag arrangement of the spores is very evident. However, he makes no mention of the genus *Beauveria.*

Finally the whole subject was brought to the field of the medical mycologist, which justifies our interest in these forms, when Langeron (**1934**) reported on his study of a fungus isolated from a case of infection of the eye in a 17 year old girl from Cairo, Egypt (case of Dr. Elia Lichaa). Langeron stated that the fungus had all the characteristics of the genus *Beauveria.* He gave a careful description such as to leave no doubt that he was indeed dealing with a *Beauveria,* but he stated that it differs in some respects from the described species. For one thing it grows at 37° C, whereas the others will not. Langeron considered it a new species and gave it the name *B. Brumpti.* However, upon the publication of Limber's paper Langeron noted the similarity of the illustrations of *Tritirachium dependens* to his *Beauveria Brumpti* and transferred his organism to the new genus, along with all of the other strains of *Beauveria* reported as human pathogens. There seems little doubt that *Tritirachium* differs from *Beauveria* in having a more constant verticillate arrangement of the sporophores, which are long and slender, not swollen, but with a thick prominent wall, distinguishing the conidiophore from the other hyphae, much as in certain *Aspergillus* species.

To further complicate the picture, Langeron states that Saito (**1939**), in Japan, had encountered this type of condiophore and created for it the genus *Spirotrichum* with the type species *S. purpureum.* Van Beyma (**1942**), in studying a similar organism, eliminated the genus *Spirotrichum* on the basis that Saito had not given a Latin description and adopted the genus *Tritirachium* Limber (**1940**), naming his culture *Tritirachium roseum* Van Beyma. Van Beyma added three species to the genus, Saito's and two of his own, making a total of eleven.

The fungi which form the basis of this study were isolated from the larvae of the beetle *Nacerda melanura* by Dr. B. O. Dodge. The larvae were obtained from wood from piles at Whitehall Street Ferry Terminal, New York City. In a recent publica-

tion, Dodge (1951) reported his findings, stating that in addition to several common saphrophytes, such as *Mucor, Aspergillus, Trichoderma* etc., he had obtained two strains of fungi which were parasitizing the larvae, one chromogenic, the other non-chromogenic. He placed them both in the genus *Beauveria.* This is believed to be the first report of such parasites from the beetle *Nacerda melanura.* Further studies were indicated for a more precise species identification and a clearer understanding of the host-parasite relationship between these piling borers and the fungus pathogen.

MATERIALS AND METHODS

The two strains isolated by Dodge (1951) designated as ✻1 chromogenic and ✻2 non-chromogenic were studied both as mass isolated strains and as monospore strains. For comparison of morphology a strain of *Spicaria* isolated from the same source was examined. In addition representative strains of each of the four species mentioned above and one of *Tritirachium dependens* were studied. The sources of these strains and the label on each when received is given below.

1. *Beauveria Bassiana* A. T. C., *Beauveria Bassiana* (Bals.) Vuill. ✻*9835,* J. H. Stevenson, U.S.D.A. W. D. Wylie
2. *Beauveria densa* A. T. C., *Beauveria densa* (Link) Vuill. ✻*9432,* Johanna Westerdijk C. B. S.
3. *Beauveria densa* C. B. S., *Beauveria densa* (Link) Vuill. fr. Halticid *760,* 51134
4. *Beauveria effusa* A. T. C., *Beauveria effusa* (Beauv.) ✻*9453,* Johanna Westerdijk C. B. S.
5. *Beauveria globulifera* C. B. S., *Bauveria globulifera* (Speg.) Picard *763,* 51137
6. *Tritirachium dependens* Strain PQMD 9–2, isolated at Harvard University, 1944, from side leather of shoe from Bougainville Island. Identification by W. L. White

We are indebted to Miss Julia Coffey of the Division of Laboratories and Research, New York State Health Department, for these cultures.

The cultures were studied on a variety of media for gross characteristics and in hanging drop and slide cultures. The slides were examined at weekly intervals for 4 weeks. Teased preparations from plate cultures were also examined. The following media were used in these studies:

Sabouraud's dextrose agar (Difco)

Potato agar with and without dextrose

1. Pared-potato water 300 gm potato 500 ml distilled water	Boil potatoes in water until mealy, filter through cheesecloth, make up to volume; for dextrose agar add 20 gr dextrose.
2. Agar water 20 gm agar 500 ml distilled water	Heat to dissolve; add to (1), tube and sterilize 25 minutes at 15 lbs pressure; pH as made up = 5–6.

Potato plugs

Casein hydrolysate medium (C-50)

$MgSO_4 \cdot 7H_2O$	0.18	grams per liter
Dextrose, C. P.	40	grams per liter
Sorensen's N/15 phosphate buffer mixture to give a final pH of 7.0	100	ml per liter
"Purified" agar	20	grams per liter
Casein hydrolysate "vitamin free"	0.5	percent
Distilled water to make	800	ml

Czapek agar (Dox and Thom's modification)

Agar	20.0	gm
Sucrose C. P.	30.0	gm
$NaNO_3$	2.0	gm
$MgSO_4$ crystals	0.5	gm
KCl	0.5	gm
$FeSO_4$ crystals	.01	gm
K_2HPO_4	1.0	gm
Distilled water	1000.	ml

Add agar to 900 ml water (hot), melt in autoclave (10 minutes 15 lbs pressure); add other ingredients one at a time in order mentioned, dissolving each chemical in turn, before adding next one. Dissolve K_2HPO_4 in 100 ml water (cold) and add slowly to agar mixture, with vigorous stirring. Distribute in small flasks. Sterilize by autoclaving at 15 lbs pressure for 15 minutes. No pH adjustment necessary. Acid reaction.

Corn meal agar

Corn meal 62.5 gm
Water 1500 ml

Heat at 60° C for 1 hour, filter through paper and make up to 1500 ml, add 19 gm agar, heat in Arnold sterilizer for 1¼ hours, filter through cotton and tube. Sterilize in the autoclave for 25 minutes at 15 lbs pressure. pH as made up is about 6.8 to 7.

Wort agar (Difco)

Sections through the mummified larvae were likewise studied both from those where infection occurred spontaneously and from one in which the infection was produced experimentally by Dodge, who sprayed an apparently healthy larva with a suspension of spores.

EXPERIMENTAL RESULTS: PARASITIC STAGE

The infected larvae appear sick and die in a short time, and almost immediately become covered with a dense coating of a white mold. This white covering, quoting Petch (1924), is what gave the name *"calcino,"* meaning chalky, to the silkworm disease in Italy and *"la muscardine"* to the same disease in France, the latter from the supposed resemblance to a sugared almond. Several transfers from this white coating yielded what appeared to be pure cultures of the same fungus. Sections through the mummified larva showed the entire larva to be filled with a mass of branching hyphae (FIG. 1 A, B). The tissues of the larva have been practically replaced by the fungus. These hyphae were much thicker than those on the surface of the larva, about twice the diameter. On the outer edge of the external mycelial mass one notes the formation of the spherical clusters or glomerules of spores that are characteristic of this fungus (FIG. 1 D, E).

Balsamo (1835), who made careful studies of Bassi's culture, was successful in artificially infecting silkworm larvae. He noted that the infected worms took on a red color, and when this red material was teased out from the larvae and examined, it was found to consist of mycelial threads and spores. By planting fragments of this material he obtained the fungus which he named

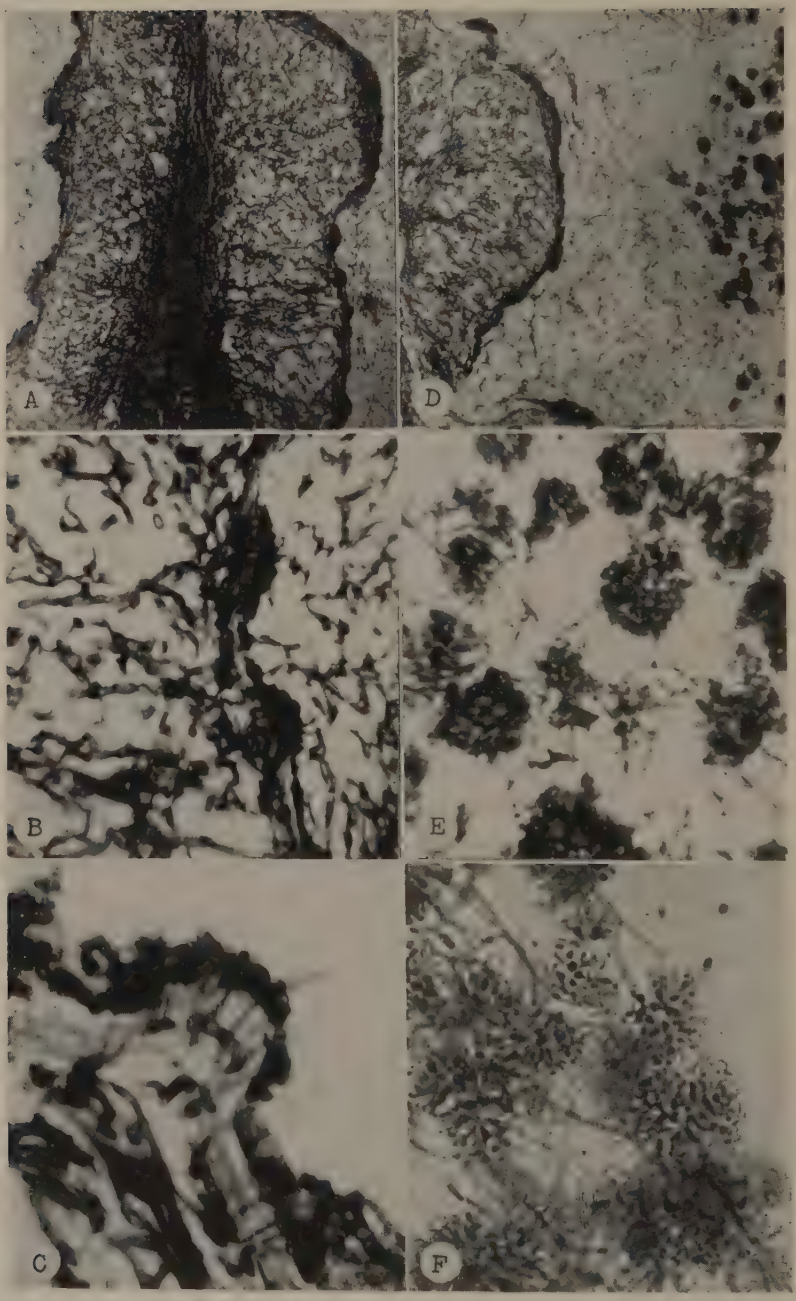

FIG. 1.

Botrytis Bassiana. Balsamo noted that the fragments of threads which he teased out from inside the dead insect were thicker than the *Botrytis* filaments. This agrees with our finding. The presence of the red pigment is significant, since later authors distinguish between a red and white muscardine. *Beauveria Bassiana* is given as the cause of the white muscardine, and other species, such as *effusa* and *densa,* of the red. As our study progressed, we were forced to conclude that the earlier workers placed more emphasis on pigment production than is warranted, since it is such a variable factor.

It is believed that these larvae are infected through the integument rather than through the intestinal tract. Picard states this, claiming that the infection is rapid, the worm dies and on the mummy are produced the external hyphae and spores which form the chalky white coating so characteristic of the disease. Extension of threads from the inside to the outer surface were noted in one of our sections (FIG. 1 C). Further studies of the parasitic stage of this group of fungi is indicated in order that we may better understand and distinguish the various species of this interesting genus.

CULTURAL STUDIES: GROSS CHARACTERISTICS

The strain which we designated as Dodge's №1 chromogenic formed at first a flocculent, cottony growth in Sabouraud's dextrose which soon became covered with a powdery mass, whereas the strain designated as Dodge's №2 non-chromogenic developed a flat, downy to felt-like white growth. They both showed a slight orange pigment on the reverse of the colony. On Potato plugs strain №1 formed a similar flocculent growth and turned the potato an intense purple red, while strain №2 produced a flat

FIG. 1. Sections through infected larva. A. Portion of longitudinal section showing the entire tissue of the larva replaced by fungus filaments, × 90. B. Outer edge of worm at higher magnification with coarse filaments within, finer ones on outside, × 450. C. Enlarged view of a portion of outer edge showing hyphae passing through integument, × 850. D. Clusters of spores which have formed on the fine external hyphae, × 90. E. High power view of clusters, × 450. F. Similar clusters from culture, × 650.

downy growth, somewhat wrinkled, but with no pigment. Monospore strains of these two isolates gave the same characteristics as the original culture. On potato plugs the chromogenic strain developed finger-like processes, so-called "clubs," or coremia (FIG. 5 A). These were never seen in the non-chromogenic strain.

On potato dextrose agar a marked difference again is noted between the two cultures; strain #1 forms in concentric rings a flat chalky powdery or granular growth, which cracks as the culture

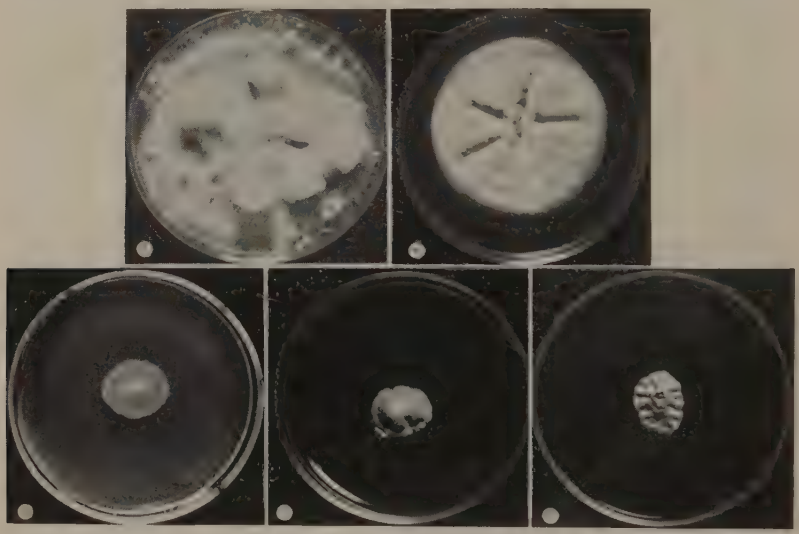

FIG. 2 A, B, E. Three-weeks-old Sabouraud's dextrose cultures of Dodge No. 1 chromogenic strain. A. Dodge No. 2 non-chromogenic strain. B, and *Tritirachium dependens*, E. C, D. Response of *B. densa* strain No. *1283* to thiamine. Cultures two weeks old on 0.5% casein medium, C, and on 0.5% casein medium plus 0.1% thiamine hydrochloride, D.

ages. A slight pink-rose pigment is formed both on the surface and on the reverse of the colony (FIG. 3 E). Strain #2 produces downy to fluffy white aerial mycelium (FIG. 3 D). The reverse shows a slight cream-yellow to rose tinge. On potato agar minus dextrose the growth is similar to texture in both instances but not so abundant (FIG. 3 A, B).

On the casein hydrolysate medium the growth of the two strains was not so easily differentiated, as both strains initially formed

a white downy to cottony growth. Later on, however, strain ✻1 developed the powery masses (FIG. 3 J). The pigment on the reverse of the colony of the chromogenic strain was a deep rose or purple whereas the non-chromogenic strain formed a rose buff pigment.

On Wort the growth is similar for each, with strain ✻1 showing a stronger pigment on the reverse of the colony.

The flat, powdery, or chalky growth is noted for the chromogenic strain on Czapek's medium with the reverse of the colony a dull rose brown, whereas the non-chromogenic race gave a more fluffy growth and a yellow pigment on the reverse.

The growth was much reduced in both instances on corn meal agar. With strain ✻1 the growth is partly submerged and transparent with scattered zones of powdery, aerial mycelium, indicative of a massive spore formation. A slight rose pigment on the reverse was noted when the strain was first isolated but later was lost. Strain ✻2 also shows a partly submerged growth and no pigmentation on the reverse.

MICROSCOPIC MORPHOLOGY

Both of our strains produce a fine, branching, septate mycelium with flask-shaped sporophores or "phialides." The conidiophores may be borne along the hyphae singly, in pairs opposite each other, or in groups. They often occur in whorls or verticils. The phialide becomes prolonged into a filament upon which the spores are borne basifugally. A spore is borne on the axis of the sporophore attached to a tiny sterigma which terminates the axis at that point. The sporophore then grows in the opposite direction producing a second spore, then in like manner a third and fourth until a zigzag arrangement is obtained with the youngest spore at the tip. The older spores may become dislodged but the sterigmata remain attached to the axis (FIG. 4 C). With the chromogenic strain it is usual to see two spores remaining attached at the tip (FIG. 4 F) whereas with the non-chromogenic there is usually only one (FIG. 4 G). In the chromogenic strain the conidiophores occur more often in whorls or verticils and the spores are numerous, many remaining attached for some time

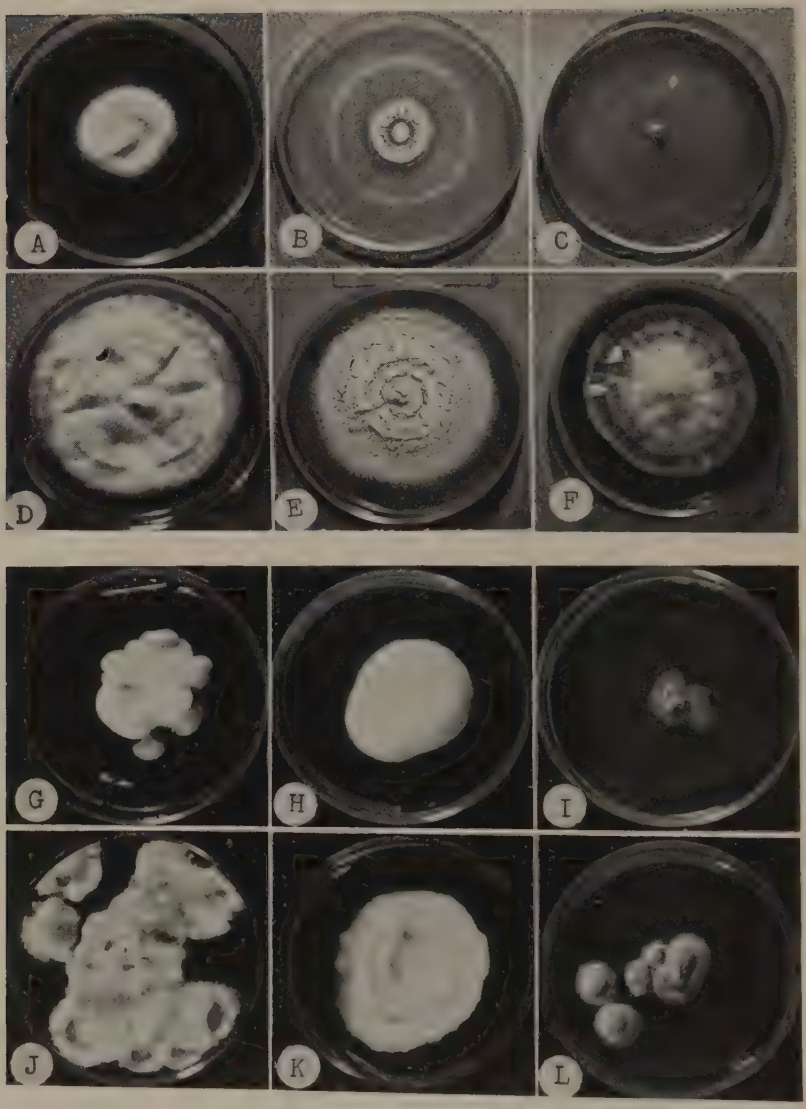

FIG. 3. Upper half. Three-weeks-old cultures of Dodge No. 2 non-chromogenic strain, Dodge No. 1 chromogenic strain, and *Spicaria* on potato agar, A, B, C, and the same on potato dextrose agar, D, E, F. Lower half. Three-weeks-old cultures of six species of *Beauveria* on casein hydrolysate medium; G. *B. Bassiana*, H. *B. effusa*, I. *B. densa*, J. Dodge No. 1 chromogenic, K. Dodge No. 2 non-chromogenic, and L. *B. globulifera*.

so that dense spherical clusters or glomerules are formed. The masses of spores give the pink powdery appearance to the cultures. Similar spherules occur on the surface of the larvae (compare FIG. 1 E, F). This powdery appearance is not found in strain №2, as there are no large spherical clusters or spores formed. Numerous spores are produced, however, but on scattered conidiophores. The branched hyphae bear many flask-shaped sporophores along the sides, often opposite each other. The swollen base of the sporophore is drawn out and usually one spore remains attached. The drawn-out filament has a curved appearance but a pronounced zigzag is difficult to see (FIG. 4, D, G). Variation in the sporophore are common in each strain.

Size and shape of spores has been used to differentiate species of *Beauveria.*

The spore measurements for our two strains are within the limits of species variation. They tend to be ovoid, though in both strains some spherical spores may be found. In strain №1 spores range from 1–2.3 × 2.3–3.3 μ, and in strain №2 spores from 1–2 × 1.5–3 μ are to be found. These measurements were made from corn meal and Czapek agar by means of the camera lucida and oil-immersion lens and did not seem to vary much with the medium. Because of the type of arrangement of the sporophores or conidiophores which predominates it is easy to distinguish between our two strains microscopically (FIG. 4 D, H).

The species of *Spicara* studied was easily identified by the chains of spores borne on phialides and by the gross appearance of the culture, which was a grayish brown color and fluffy in character (FIG. 3 C, F).

OTHER BEAUVERIA SPECIES

Of the other *Beauveria* species listed above all were shown to have the characteristic sporophore (FIG. 4 B, I, J, K).

It would be very difficult to distinguish the strains of *B. Bassiana* and *B. effusa* studied from each other or from our chromogenic strain, either from their gross characteristics or the morphological structure. The two strains labelled *B. densa* are similar to one another, but differ from the other strains in that they have

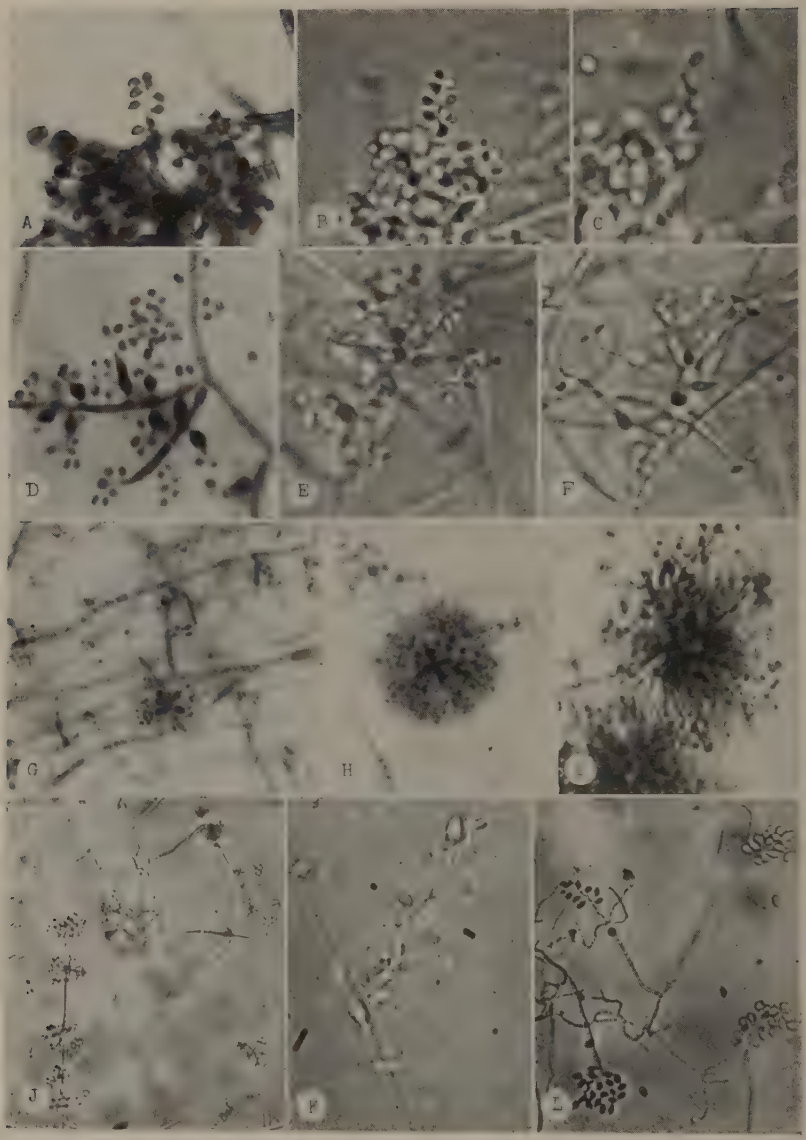

FIG. 4. Photomicrographs from slide cultures. A, B. Edge of spore clusters showing zigzag arrangement of spores: A, in section from infected larva; B, from slide culture of *B. Bassiana*. × 1450. C. Conidiophore of Dodge No. 1 chromogenic strain showing sterigmata to which spores were attached, × 2000. D, G. Characteristic conidiophores of Dodge No. 2 non-chromogenic strain. G, a young cluster, × 400. D, × 1450. E, F. Dodge

somewhat larger and more distinctly elongated spores. The conidiophores are not swollen, but long and slender (FIG. 4 K). Another interesting point which serves to distinguish these two organisms from the others under discussion is the fact that they appear to have a vitamin deficiency. It was noted that on the casein hydrolysate and the Czapek media, neither of which contain vitamins, the *densa* strains failed to grow or produced only a slight growth (FIG. 3 I). When thiamine hydrochloride was pipetted over the surface of these plates, or added to the medium, growth increased and soon equalled that of the other strains. This would seem to indicate that the *densa* strains require thiamine for optimum growth (FIG. 2 C, D).

B. globulifera showed a similar though less pronounced deficiency for this vitamin (FIG. 3 L). This strain differed in its flat, downy, more compact growth, in the yellow color of the aerial mycelium, and in the deep orange diffusible pigment which formed on the undersurface of the colony. It colored the potato a greenish-yellow rather than red; this has been reported by others for this species. *B. globulifera* according to earlier workers resembled in its microscopic structure both *B. bassiana* and *B. effusa*. Picard (1914) illustrated under the name *B. globulifera* the glomerules found to be so characteristic for our chromogenic strain and the one which we received as *B. bassiana*. The dense, closely-matted growth of the strain of *B. globulifera* studied obscured the individual spherules, but that they were formed is well illustrated in FIG. 4 J. Variation between the flat, more compact type of growth and the flocculent or cottony type may be noted in a given strain. This was well demonstrated by our strain of *B. bassiana*. A Sabouraud-dextrose plate culture developed in two sectors, one flat, the other more flocculent. Transfers were

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No. 1 chromogenic strain, two optical levels of a spherical cluster, lower level showing swollen bases of phialides, E, and F showing the terminal zigzag, × 1450. H. Same strain showing fully formed spherical clusters, × 400. I. Clusters of *B. Bassiana;* note zigzag conidiophore similar to F, × 730. J. Young clusters of *B. globulifera,* × 300. K. conidiophore of *B. densa,* showing more oval spores and no swollen base, × 1450. L. Conidiophore of *Tritirachium dependens* showing similarity to *Beauveria,* × 650.

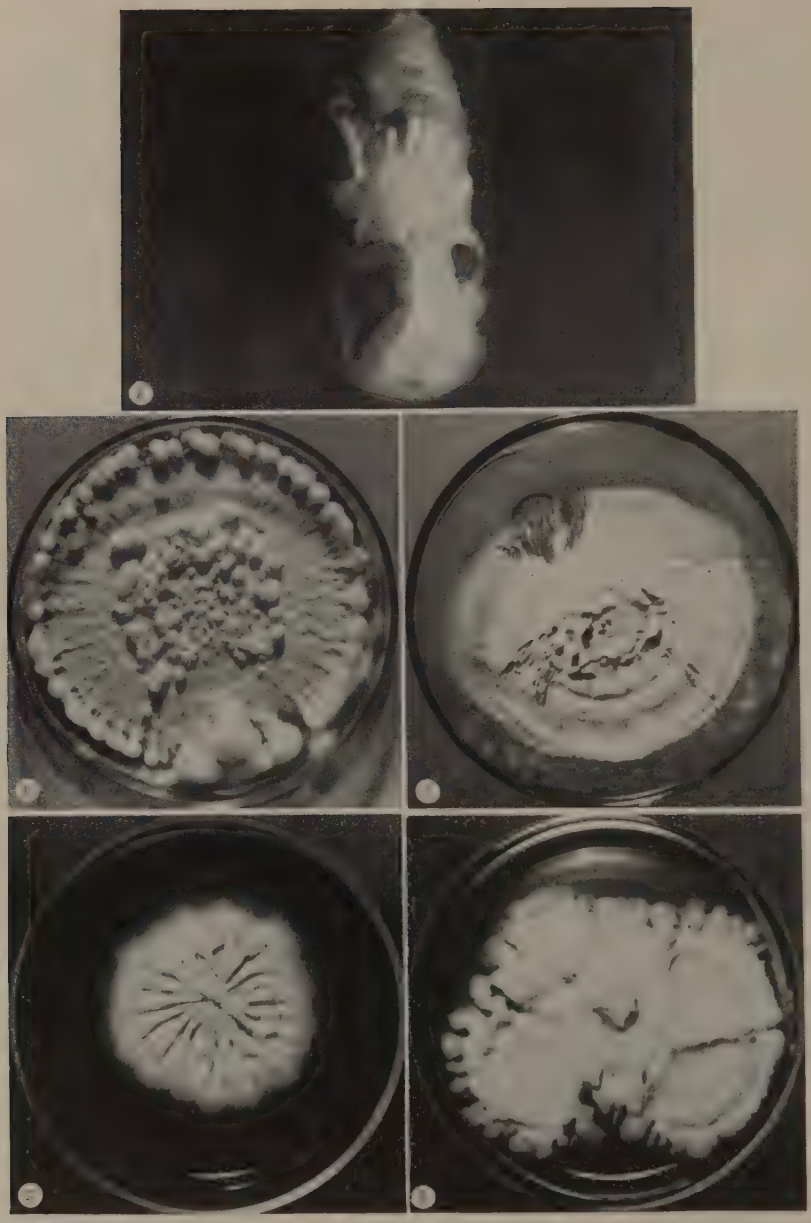

FIG. 5. Cultures of Dodge No. 1 chromogenic strain and *B. Bassiana*. A. Potato agar plug culture of Dodge No. 1 chromogenic showing coremia and dark patches of pigment. B. Three-weeks-old culture of *B. Bassiana*

made from each sector to wort agar plates with the following results: the flat sector resulted in a folded, downy colony and the more flocculent sector developed a fluffy, cottony growth with characteristic coremium formation (FIG. 5 B, C, D, E). These characteristics were maintained through repeated transfers. Monospore strains of these two types should be obtained and observed for further variation; nevertheless, this observation serves to point out the variability of the gross appearance of this group of micro-organisms.

TABLE I

COMPARISON OF STRAINS OF BEAUVERIA STUDIED

Strain	Predominant type of growth on Sab. D	Coremia	Pigment	Spore measurements	Predominant types of conidiophore
B. Bassiana	Flocculent or wool-chalky, cream	+	Slight	1–1.7 ×1.2–2.5μ	Spherical clusters
B. effusa	Downy-cottony, white	+	Not noted	1.2–2 ×1.3–3μ	Spherical clusters
Dodge Chromogenic	Flocculent or wooly, chalky	+	+	1.2–3 ×2.3–3.3μ	Spherical clusters
Dodge Non-Chromogenic	Flat, compact, downy-white	–	–	1–2 ×1.5–3μ	Scattered phialides, few in verticils
B. densa (1)	Downy-white	+	Not noted	1–3 ×2.6–4.3μ	Phialides reduced
B. densa (2)	Downy-white	+ Slide culture	Not noted	1.5–2 ×2–4.8μ	Phialides reduced
B. globulifera	Compact, felt-like, firm, cream to yellow	Not noted	Yellow to greenish to orange	1–2.1 ×1.5–3.3μ	Compact mass of phialides

Tritirachium dependens is distinct both in gross and microscopic appearance. On Sabouraud's agar a compact, felt-like, convoluted tan colony forms with a deep purplish brown color on the undersurface (FIG. 2 E). The conidiophore, as noted above, is long and slender with spores borne in a zigzag arrangement (FIG. 4 L). The swollen phialide-like structure is lacking.

on wort agar showing coremium formation. C. *B. Bassiana* on Sabouraud's dextrose agar showing sectors. D, E. Transfers from sectors to wort agar, D from upper, flatter sector, E from lower, more fluffy sector. Note similarity of E to B, the parent culture.

The characteristics of the above-named species and our new isolates are summarized in TABLE I.

DISCUSSION

It will be noted from the above table that our chromogenic strain is very close to *B. Bassiana* and *B. effusa*. Beauverie long ago noted the close relationship of these two species. He differentiated them primarily upon the gross characteristics, a flocculent versus a chalky growth, and red pigment versus no pigment. However, as we have seen, the strain of *B. Bassiana* here studied has a flocculent type of growth and a slight pigment production, whereas the *B. effusa* strain has a downy growth and no pigment production. As pigment production has been noted by many to be a variable factor and to diminish or become entirely lost with time it would not seem a sound basis for differentiating species. Also there is no assurance that the strains now existing under the name *Bassiana* are indeed identical with that isolated by Bassi. Balsamo, who studied the original strain, noted the red coloration in the infected larvae. It is only after death that the mummy becomes covered with the white coating. Beauverie claims that with the red muscardine the color remains red. The larvae which we examined were covered with a white coating.

The spore measurements are within the limits of variation for the three species. It would seem that our chromogenic strain is definitely *effusa* and it certainly is indistinguishable from the strain which we studied under the name *Bassiana*, except in pigment production.

Our non-chromogenic strain might well answer to the description of the original *Bassiana* species in the type of growth and lack of pigment, in which characteristics it differs from the above three strains. The strain of *B. globulifera* studied has certainly not the macroscopic characteristics as first described for that species but variation in the type of growth for a given strain is common. The tendency to form a yellow pigment has always been attributed to this form and we have noted formation of glomerules. The two *densa* strains seem distinct both in spore size and shape, and in the slender conidiophores. The vitamin deficiency shown

by these two strains would also indicate a difference. We believe that *densa* is a distinct species, but that further studies should be made with the other strains here listed, particularly from the standpoint of the type of infection they cause and their virulence. Perhaps in this way a more definite conclusion might be reached as to both the number of distinct species involved and the variation that may occur within a single species.

Limber's strain is a distinct species and may be identical with Langeron's *B. Brumpti.*

SUMMARY

Two strains of *Beauveria* isolated from the wood borer *Nacerda melanura* were studied and compared with named species. The chromogenic strain appeared to be closely related to if not identical with the strains of *B. Bassiana* and *B. effusa* studied. Whereas the non-chromogenic strain seemed similar to the published descriptions of *B. Bassiana,* the two strains of *Beauveria densa* were identical, and are believed to constitute a distinct species. The strain of *B. globulifera* appeared different from the published descriptions of this species. It is, however, probably closely related to the *Bassiana* group, as it has been demonstrated that variation in growth characteristics may occur. Further studies are necessary, especially of the pathogenicity of these strains, before conclusions as to species differences can be drawn. A critical study of a number of species of *Tritirachium* should also be made in order to determine the validity of the genus. Sections of the infected larvae were studied and described.

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THE COPRINUS EPHEMERUS GROUP

Morten Lange and Alexander H. Smith

For some time we have been working on black-spored agarics, and on some problems involving the geographical distribution of these species. During this study we found it necessary to clear up the relationships between the American and the European species, eventually by studying them in culture. Special concern was given to the possible interfertility of American and European strains, and its bearing on our species concepts.

The Danish author undertook the necessary culture work, and found each American strain he raised to be interfertile with a group of European interfertile strains. The intrafertile groups thus defined were found to correspond to rather narrowly delimited species. A more detailed account of this work, including a discussion of the species concepts arrived at, is published elsewhere (Lange 1952).

In the light of this study we venture to publish this contribution to a monographic treatment of the group in the hope that it will furnish those working in other parts of the world with species concepts which, when applied to the problem of distribution, will help to make that study more meaningful than has been the case in the past. A few species originally thought to belong here, but since excluded after a detailed study of their morphology, are included for convenience.

SURVEY OF DIAGNOSTIC CHARACTERS

The subsection is here in the main as defined by J. Lange (1915, 1939) using the absence of a universal veil and presence of pilocystidia as distinguishing characters. The species are found in two main habitats: on manure and on humid, black forest soil and mud. This difference in ecology proved to be a valuable and quite reliable diagnostic character, dividing the subsection into

a group of coprophilous and one of terrestrial species. The morphological characters show a rather great variability within each species, and both size and color can be used as distinguishing characters only after careful study. The size of the pileus is here in general recorded as the height just before expansion begins. The length and width of the stipe is taken on the fully mature fruit body. Ridgway (1912) was used as the standard for recording colors. It was found, also, that the microscopic characters vary a great deal, but still provide several important criteria of diagnostic value. Spore size and shape have been recorded with special care, and in addition to the usual maximum and minimum values we have generally indicated the variation in average size, as found by measuring a great number of spores of each of the available collections. The average ratio between length and width is a figure of some importance and is recorded for most species $\left(\frac{D}{d}\right)$. Almost all species have the spores slightly compressed. We have indicated this by figures for the difference between the two short diameters (d_1–d_2).

The basidia are dimorphic in some species, tri- or tetramorphic in others. Two-spored basidia have been found characteristic of some species, although a further study may reveal four-spored corresponding forms.[1] The presence or absence of pleurocystidia is an important character, especially in the coprophilous group, whereas differences in cheilocystidia often separate the species in the terrestrial group. Both pleurocystidia and cheilocystidia are best observed on buds which are on the point of expanding. Pilocystidia are always abundant on young buds and are of great diagnostic value. Their size is here recorded by the measurements of length, width at broadest point at base, and width just below apex (as close to apex as the sides can be considered to be subparallel). There are two types, one hyaline and thin-walled and the other with thick walls which vary from hyaline to brown. The two kinds intergrade. Such thick-walled bodies are often termed "setae," but they are truly pilocystidial in origin. We

[1] In the one case where two such corresponding forms were found to exist (*C. bisporus* and *C. congregatus*) they were found to be intersterile (Lange, 1952) and hence are not regarded as identical.

prefer to designate them as *sclerocystidia* to differentiate them from the thin-walled type.

Clamp connections are present in all terrestrial species and in several of the coprophilous group. They are frequently hard to demonstrate on the fruit body, but form one of the best cultural characters, since they are easily demonstrated on the mycelium. Few other mycelial characters are of general importance, although great weight should be placed on the pairing reactions of the species. Both homothallic and heterothallic, bipolar and tetrapolar types are found within the subsection. Some of the species are amphithallic (Lange **1952**). In these species spores from the same cap give rise to both homothallic and heterothallic single-spore mycelia.

Strains in the same interfertility group will as a rule all be fully interfertile. Identical allelomorphs were, however, found in several cases, and also a few examples of reduced interfertility, due to other factors, but never correlated with any significant morphological differences.

TAXONOMY

As the subsection Setulosi (J. Lange **1915**) is used here it can be covered by the following amended description: Small to medium sized species; pileus fisso-sulcate or deeply grooved; no universal veil; partial veil absent (or rudimentary ?); deliquescing readily or tardily; pilocystidia present; pleurocystidia present or absent; cheilocystidia balloon-shaped or fusoid-ventricose; basidia di- or pleomorphic; spores brown to black (reddish in one species), generally more or less flattened; clamp connections present or absent.

The type species of the subsection is *Coprinus ephemerus*.

Singer (**1951**) has vaguely defined a subsection Auricomi, characterized by the presence of "setae" on the cap. Its type species, *C. auricomus* is not very well known, but the subsection is probably synonymous with Setulosi.

The true members of Setulosi form a group of closely related species which are frequently difficult to distinguish. We have arranged the species in stirpes, uniting those most closely related to

each other in small groups and arranging the whole in a sequence beginning with the most coprinoid, strongly deliquescing taxa. Those which are included here from other subsections and characterized by the presence of a universal veil made up of sphaerocysts apparently do not form a homogeneous group, and have here been arranged together with those of the Setulosi to which they seem to be most closely related.

KEY TO SPECIES

1. Coprophilous species, also growing on rotting straw; rapidly deliquescing....2
1. Habitat not as above....10
2. Sphaerocysts and clamp connections present; spores hexangular *C. hexagonosporus*
2. Not as above....3
3. Sphaerocysts present, clamp connections absent; germ pore eccentric; spores narrow (5.2–7.5 μ), (if spores 7–9 μ broad in broadest view and buttons reddish see *C. curtus*)....*C. heptemerus*
3. Sphaerocysts absent on buttons....4
4. Clamp connections absent....5
4. Clamp connections present....8
5. Larger forms (buttons > 0.8 cm. high); spores medium to large (average length > 11.5 μ), germ pore eccentric....6
5. Small forms (buttons < 0.8 cm. high); spores small to medium sized (< 11.5μ), germ pore almost apical....7
6. Basidia 4-spored; pileus clay color to ochraceous tawny *C. congregatus*
6. Basidia 2-spored; pileus pale ochraceous tawny to ivory white *C. bisporus*
7. Spores broad (D : d < 2); pleurocystidia present....*C. stellatus*
7. Spores narrow (D : d > 2); pleurocystidia absent....*C. pellucidus*
8. Spores long, average > 11.5 μ; pleurocystidia present....9
8. Spores short, average < 11.5μ; pleurocystidia absent *C. heterosetulosus*
9. Basidia 2-spored; thin-walled pilocystidia narrow, thick-walled pilocystidia (sclerocystidia) frequent....*C. Sassii*
9. Basidia 4-spored; thin-walled pilocystidia inflated; thick-walled pilocystidia (sclerocystidia) rare to absent....*C. ephemerus*
10. Carbonicolous species. Spores mitriform; pleurocystidia frequent; clamp connections present....*C. angulatus*
10. Terrestrial to sublignicolous species; pleurocystidia absent in many; clamp connections present; often tardily deliquescing....11
11. Spores vinaceous brown when fresh, turning pale chocolate color in KOH; pileus vinaceous brown in buttons....*C. fallax*

11. Not as above, spores typical for genus.....12
12 At least most of the cheilocystidia subglobose.....13
12. Cheilocystidia mostly bottle-shaped.....17
13. Pilocystidia subcapitate.....*C. plagioporus*
13. Pilocystidia obtuse to acute.....14
14. Spores long ($>11.5\,\mu$), germpore eccentric.....15
14. Spore medium ($<12\,\mu$), germpore eccentric to almost apical....16
15. Pileus and stipe flushed purplish; spores narrow (D:d > 1.9), almost black and impellucid.....*C. subpurpureus*
15. Pileus pallid to brown; spores paler; sclerocystidia on pileus frequent.....*C. sclerocystidiosus*
15. Not as in either of above choices—see *C. callinus,* the large spored forms.
16. Pilocystidia long ($>90\,\mu$), mostly acute.....*C. callinus*
16. Pilocystidia shorter ($<90\,\mu$) mostly obtuse.....*C. subimpatiens*
16. Pilocystidia short and acute, see *C. eurysporus.*
17. Pilocystidia short, average $<60\,\mu$.....18
17. Pilocystidia longer, average $>70\,\mu$.....19
18. Spores in face view almost as broad as long, average $<11\,\mu$ *C. eurysporus*
18. Spores longer in relation to widest view, average $>12\,\mu$ basidia 2-spored.....*C. amphithallus*
19. Fruiting body small; pilocystidia and cheilocystidia obtuse; spores $>10.5\,\mu$ long.....*C. subdisseminatus*
19. Fruiting body larger (buttons >0.7 cm. high); cheilocystidia subacute; spores $<10.5\,\mu$ long.....20
20. Pilocystidia average $<90\,\mu$; spores ovate, apiculus small.. *C. impatiens*
20. Pilocystidia long ($>110\,\mu$); spores narrow (D:d > 1.9), apiculus prominent.....*C. hiascens*
20. Spores average $<8\,\mu$; stipe and gills brown.....see *C. digitalis*

Section FARINOSI

1. Coprinus heptemerus nom. nov.

Coprinus curtus f. *macrosporus* Romagnesi (1941).

Illustrations: Lange 1952, Figs. 2, 9.

Pileus less than 1.0 cm high, subcylindric, narrow, rather pale, dull brown, pale "clay color," fissosulcate, deeply plicate when expanding, finally splitting at the edge and recurving; flesh very delicate and thin, odor and taste not distinctive; lamellae rather narrow and distant; stem whitish to hyaline, slightly brownish at the base; deliquescing.

Spores 10.8–15 × 4.4–7.5 μ, average [2] 11.5–13.9 × 5.2–6.7 μ, narrow, $\frac{D}{d} = 1.9–2.3$, somewhat flattened, $d_1–d_2 = 0.2–0.6$ μ, irregularly cylindric to narrowly ovate, rather pale chocolate color, suprahilar depression rather pronounced; germpore almost fully visible on abaxial side, often very large, up to 4 μ broad; basidia dimorphous, 20–33 × 10.5–13 μ, four-spored; paraphyses globose, about 18 μ broad; pleurocystidia absent; cheilocystidia balloon-shaped, about 25–50 μ broad; gill trama mostly of inflated elements, with an inner layer of fibrillose cells; pileus trama with a cuticle formed by a row of vesiculose cells mostly 20–30 μ broad; pilocystidia 50–122 × 9–23 × 2.5–4 μ, much inflated below, acuminate, sometimes furcate, hyaline, some incrusted, intergrading into large, globular, incrusted sphaerocysts; clamp connections absent in all tissues and on the mycelium, which in culture has a very pronounced dark brown color.

Homothallic, fruiting abundantly in culture within seven days after inoculation.

Habit, habitat and distribution: Subsolitary on dung, France *, Denmark *, Sweden * and U.S.A.*

Observations: This fungus was first described by Romagnesi (1941) as a form of *C. curtus*, from which it is clearly distinguishable by the lack of reddish colors, acuminate cystidia, pale spores with strongly eccentric germpore and several other characters. When the sphaerocysts are washed away it bears some likeness to *C. stellatus* and *C. pellucidus* but it is probably not intimately related with the species in the section Nudi. We have seen several collections by Brooks from Michigan, it has been found twice in Denmark and twice in Sweden, and we have checked the authentic culture of *C. curtus* f. *macrosporus* in Baarn. The latter was found to be identical with our own material in all details and should be considered as the type.

[2] Indicates the smallest and the largest average spore size found when measuring spores from several different collections. For many species a very large variation is recorded.

* The asterisk indicates that specimens from that country have been cultured and in heterothallic species found interfertile with other strains of the species.

2. COPRINUS HEXAGONOSPORUS Josserand (1948).

Illustrations: Josserand 1948, Fig. 1; Smith 1948, Fig. 9; Lange 1952, Figs. 1, 9.

Pileus cylindric at first, 0.5–2.0 cm high, very finely pubescent over disc, disc "fuscous," marginal area pallid to "avellaneous," at maturity the disc often tinged "army brown," surface closely folded-striate before expanding, becoming broadly convex to plane and plicate-striate before maturity; flesh very delicate and thin, odor none, taste mild to slightly bitterish; lamellae free but attached at apex of stipe, close, becoming subdistant, whitish pallid, soon avellaneous and then black, narrow and equal, soon deliquescing; stipe 3–5(–10) cm long, 0.5–1(–2.5) mm thick, equal, hollow, very fragile, sparingly pubescent at first from projecting caulocystidia, densely pubescent above, white or whitish, hyaline at first but soon sordid brownish over lower two-thirds, base often inserted on the substratum.

Spores black in deposit, 8.6–14.5 × 5.4–8.3 μ, aver. 10.9–12.6 × 6.05–7.3 μ, distinctly flattened, d_1–d_2 = 0.75–1.15 μ, subelliptic in side view, angular in face view (the sides parallel and angled to apex and base), pore distinctly to very much eccentric (sometimes appearing circular in face view of spore), blackish brown, black in KOH; basidia four-spored, rarely with a few two-spored basidia intermixed on same pileus, 20–34 × 8–11 μ, more or less trimorphic; paraphyses 18–22 × 9–16 μ; pleurocystidia rare to absent, 60–90 × 20–35 μ, becoming more elongated and narrower, readily collapsing; cheilocystidia similar to pleuro-cystidia or more vesiculose, 40–90 × 35–45 μ; gill trama narrow and of very loosely interwoven cells; pileus trama very thin but hyphae filamentose, those in the dark discal area dark sordid brown (almost vinaceous brown) when revived in KOH; cuticle of vesiculose cells, pilocystidia 50–120 × 9.5 μ, inflated below, neck subcylindric, apex more or less distinctly capitate (width respectively 9.5–22 μ, 4–10 μ and 4–13μ), hyaline or slightly brownish below, typical sclerocystidia not present but pilocystidia intergrading into incrusted globose sphaerocysts on the surface, these mostly 20–30 μ broad; clamp connections present on hyphae at base of stipe and on mycelium.

Heterothallic and tetrapolar.

Habit, habitat and distribution: Solitary or gregarious on dung and rotton straw, France *, Denmark * and U.S.A.*

Observations: This very outstanding species was first mentioned by Pennington (1918) as a form of *C. ephemerus*. It is

recognizable macroscopically by its dark fuscous to vinaceous brown apex, but the microscopic characters are necessary to clearly distinguish it from *C. Sassii*. Josserand (1948) noted the sphaerocysts on the pileus. However, these are generally very scanty except on the very young bud. The species is probably the best link between the Nudi and Farinosi. The color of the pileus and type of pilocystidia indicate relationship with *C. curtus*.

Stirps *Sylvaticus*

3. COPRINUS SILVATICUS Peck (1870).

Coprinus tardus Karsten (1883).

Pileus 1–4 cm high, broadly elliptic-ovate, then broadly convex and finally almost plane, the edge sometimes recurving, finely pubescent, soon almost glabrous, fisso-sulcate to the smooth disc, disc and back of striae dark brown ("cinnamon brown" "russet"), darker when very moist, paler in some forms, appearing somewhat viscid when moist; flesh concolorous with pileus, pelliculose except in the disc, taste and odor none, tardily deliquescing; lamellae distinctly brownish when young, black when spores ripen, crowded to subdistant, narrow, adnate to almost free; stipe 1–8 cm × 0.2–2.5 mm, whitish to yellowish brown.

Spores 10.3–15.8 × 6.2–10.0 μ, flattened, d_1–d_2 = 0.6–1.15 μ, broad, D : d = 5–1.6 μ, broadly ovate or almost circular in face view, slightly inequilateral in side view, with low warts and ridges covered by a more or less distinct wrinkled hyaline epispore, apiculus prominent, blackish brown to almost impellucid, germpore apical; basidia 20–46 × 9–11.5 μ, four-spored, rarely with some few two-spored intermixed, tetramorphic, long type narrow, much protruding; paraphyses inflated, pleurocystidia absent; cheilocystidia bottle shaped, 38–64 × 16–18 × 5–8 μ, in a rather dense band; pileus trama brownish, cuticle of vesiculose cells, pilocystidia very large, 75–135 × 15–25 × 4.5–9.5 μ, sphaerocysts present but seldom numerous, 20–35 μ broad, brownish with wrinkled walls.

Heterothallic, clamp connections present on mycelium.

Habit, habitat and distribution: Subfasciculate to gregarious, on rich soil in woods and gardens. We have seen material from U.S.A., Canada, Finland, and Denmark *, but also on record under different names from several European countries in addition to the above.

Observations: This species is easily recognized by the large rough spores, the stature which reminds one of *C. micaceus,* and in somewhat the same color pattern as the latter. Various forms have been described as follows: with almost glabrous spores, with very few pilocystidia, and with pallid colors. The species is often called *C. tergiversans* Fr. sensu Ricken, but as pointed out by J. Lange (1939) the Friesian description hardly fits this plant. The concept of Peck's species is well based on the emended description of the type (Smith 1948). In addition, we have studied the type of Karsten's *C. tardus* and found it identical in all characters. The sphaerocysts are not mentioned in the current descriptions, but have been demonstrated on both American and European material. The spores of this species are very difficult to germinate, and no American material has been studied in culture. However, we feel quite certain that the American and European material studied belongs in one species on the basis of resemblance in morphological characters.

Section Nudi: Subsection Setulosi:

Stirps *Ephemerus*

4. **Coprinus Sassii** nom. nov.

Coprinus ephemerus f. *bisporus* Sass (1929).
Illustrations: Lange 1952, Figs. 2, 10.

Pileus 0.8–2(–2.6) cm high, narrowly conic, expanding to obtusely conic, the margin finally recurving and splitting, surface pruinose at first but soon almost glabrous, moist, plicate-striate to the disc but disc not differentiated by a sharp line, "russet" to "cinnamon brown" with a slight purplish flush when young, "mummy brown" when spores mature, the disc retaining a tawny tinge; flesh very thin, soft, delicate, odor and taste none; lamellae very narrow, free or just reaching the stipe, rather crowded, pallid, edge whitish but finally black over all as they deliquesce; stipe 4–8 cm long, 2–3 mm thick, hollow, fragile and very delicate, white or more frequently flushed with color of the pileus from base up.

Spore deposit black, spores 12.8–20 × 7.9–11.0 μ, average 15.2–17.3 × 8.7–9.8 μ, $\frac{D}{d} = 1.7–1.75$, almost terete, broadly ovate in

face view, broadly elliptic-ovate in side view, dark brown, blackish brown in KOH, germpore distinctly visible on abaxial side, large, about 2.5 μ broad; basidia 18–38 × 9–12.5 μ, constantly two-spored, dimorphic, the short type often considerably broader than the long one; paraphyses subglobose, 12–18 × 18–25 μ; pleurocystidia scattered to abundant, vesiculose, 50–90 × 15–55 μ; cheilocystidia similar but somewhat shorter, mostly 30–60 × 30 μ; gill trama scanty, hyphae about 3 μ broad, a few of them with clamp connections, rusty brown near cap trama which is made up of narrow elements, rusty brown in KOH; cuticle of subglobose cells with rather thick walls, diam. 20–35 μ, pilocystidia 30–100 × 4.5–16 × 1.5–5 μ generally rather thick-walled and brownish from the base up more or less, some of them typical sclerocystidia and all of them narrow, pedicellate to sessile, subventricose, acuminate, apex acute (subacute in one American coll.), the base frequently incrusted with hyaline material, more rarely pilocystidial bodies are seen without a neck, but with a long pedicel.

Single spore mycelia from the same pileus always of two types, with and without clamp connections, the former homothallic, the latter heterothallic, bipolar ("amphithallic" bipolar, cfr. Lange **1952**).

Habit, habitat and distribution: Gregarious on horse-dung and on rotten straw, U.S.A., Denmark *, Sweden *.

Observations: The material described here evidently belongs in what Sass (**1929**) called *C. ephemerus* f. *bisporus*. We have studied the type in the University of Michigan Herbarium, and found it fully identical with our collections. In addition, the cultural characters were as described by Sass. It is different from *C. ephemerus* (whichever concept of the latter one chooses to accept) by the characteristic pilocystidia and the outstanding color. In stature and color it resembles *C. hexagonosporus*. We have a single American collection, from Kansas, of a four-spored *Coprinus* probably closely related to *C. Sassii* but with more pronounced purplish colors and smaller spores. Until more collections have been made, we hesitate to consider it the four-spored form of *C. Sassii*. The two-spored basidia of *C. Sassii* are a genetically constant feature as is evidenced by their occurrence through several generations in culture. The sparingly occurring cystidia without necks might indicate a relationship to species with sphaerocysts.

5. COPRINUS EPHEMERUS Bull. ex Fr. sensu Locquin (1947).

? *Coprinus proximellus* Karsten (1879).

Illustrations: Locquin 1947, Fig. 1; Lange 1952 Figs. 2, 10.

Pileus up to 2 cm high, generally 1–1.3 cm but also much smaller, buttons narrow but becoming broadly conic, the margin recurving and splitting in age, fisso-sulcate, plicate-striate when maturing, disc pale to dark "cinnamon brown" or slightly more vividly colored, somewhat shining when moist; flesh thin, distinctly bitterish, rapidly deliquescing; gills close to subdistant, up to 36 reach the stipe, narrow, very narrow behind, almost free, pallid, blackening from the spores, edge whitish; stipe up to 7 cm long and 3 mm thick, white, slightly thickened and strigose below, hollow, caulocystidia present.

Spores black in deposit, very variable in size, 8.6–16.7 × 5.3–8.5 μ, average 11.35–15.4 × 6.1–7.6 μ, moderately broad to rather narrow, $\frac{D}{d} = 1.8–2.05$, slightly flattened to almost terete, $d_1–d_2 =$ 0.0–0.7 μ, mostly 0.15–0.5 μ, ovate in face view, elliptic-oval in side view, moderately dark brown to almost black, black in KOH, germpore more or less visible in abaxial side; basidia 20–33 × 11–13 μ, four-spored, mostly dimorphic; paraphyses subglobose-pyriform, 13–20 μ broad; pleurocystidia abundant, 60–120 × 22–45 μ, vesiculose, more or less pedicellate; cheilocystidia similar or shorter, subglobose, 20–60 × 20–30 μ, a few elongated-ventricose cheilocystidia noticed on most specimens, generally about 55 × 15 μ; gill trama scanty, of thin, sometimes clamp-bearing hyphae with additional more inflated subhymenial elements; pileus trama mostly of inflated elements, 12–14 μ broad; cuticle of globose to subglobose cells 25–30 μ across, in the grooves more oblong, and then up to 70 × 25 μ; pilocystidia 33–94 × 9–19 × 3.5–8 μ, more or less ventricose, neck subcylindric to somewhat tapering, apex obtuse, hyaline or faintly brownish below; sclerocystidia absent.

Heterothallic, bipolar, the mycelium with numerous clamp connections.

Habit, habitat and distribution: Solitary or gregarious, on cow dung, usually after the droppings have aged about a month or more and persisting at times on very old washed-out substratum. It occurs rarely on rotting straw, Denmark *, Norway *, Sweden *, France, U.S.A.*

Observations: This species is distinguished from other large-spored species of the group by the presence of clamp connections,

bipolarity, and other cultural as well as microscopic characters, but it can often be recognized macroscopically. It is evidently the species studied in culture by Sass (**1929**) and by Quintanilha *et al.* (**1941**). It was described very well by Locquin (**1947**) who noted the bitter taste and the affinity for cow dung. The "correct" application of the name *C. ephemerus* is, in our opinion, an almost insoluble problem, and perhaps the name should be discarded. Fries noted (**1874**) that the name was used for a group of species. Brefeld, Karsten and Schroeter used the name for a small-spored species, whereas Cooke and all more recent authors have used it for a large-spored species. There is little doubt that Karsten called this species *C. proximellus,* though we have not been able to demonstrate the clamp connections on his type material. Karsten's description is, however, entirely insufficient. We follow the concept of Locquin not only because we thus are in accordance with the majority of later authors, but particularly on the strength of the original Friesian description (Fries **1821**), indicating relatively large size and brown colors.

6. COPRINUS HETEROSETULOSUS Locquin (1947).

Illustrations: Locquin 1947, Figs. 2, 3; Lange 1952, Figs. 3, 10.

Pileus rarely more than 0.7 cm high, rather broad and obtuse, densely cystidiose when young, fisso-sulcate, then plicate-striate, striae frequently somewhat wrinkled, disc dark brown "Saccardo's umber," the striae more or less flushed with this color, margin appressed against the stipe at first, pale, turning blackish and splitting when spores ripen; flesh delicate, without taste, soon deliquescing; lamellae narrow, close to subdistant, often only 16 reach the stipe, almost free, pallid brownish, almost black when spores ripen; stipe about 1–3 cm long, 1.0 mm thick, hyaline whitish, flushed with the color of the pileus at the base, densely pubescent when young, hollow.

Spores almost black in deposit, 8.1–12.8 × 4.8–6.6 μ, average 9.6–11.15 × 5.4–6.0 μ, fairly narrow $\left(\frac{D}{d} = 1.8\text{–}2.05\right)$, slightly flattened to almost terete, ovate in face view, elliptic in side view, germpore slightly eccentric, hardly visible on abaxial side, dark brown to blackish brown, fuscous to black in KOH; basidia four-spored, 20–35 × 8–9.5 μ, trimorphic; paraphyses subglobose, 14–

17 μ broad; pleurocystidia absent, cheilocystidia globose, 15–30 μ across, elongated-ventricose types rare to scattered; gill trama of thin clamp-bearing hyphae, with inflated subhymenial elements; trama of cap of inflated elements, 10–30 μ broad, mixed with some thinner, dark brown hyphae; cuticle of globose cells, more oblong, up to 75 μ long in the grooves; pilocystidia 30–105(–140) × 7.5–21 × 3–6(–9)μ, often distinctly ventricose, base frequently brownish, neck somewhat tapering, apex subobtuse to almost acute; sclerocystidia rather abundant to rare, not seen in some caps, when present small, brown, thick-walled and irregular, rarely more than 33 × 8.5 × 3 μ.

Heterothallic, tetrapolar, clamp connections abundant to scattered on the mycelium.

Habit, habitat and distribution: On dung, often when rather old and washed out, France *, Denmark *, Norway *, Sweden *, U.S.A.* (Michigan and Washington).

Observations: This species is well characterized through its microscopic characters, especially when sclerocystidia are present. It is the only small-spored species in the coprophilous series with clamp connections. Locquin (**1947**) suggests that the species which Josserand (**1933**) called *C. ephemerus* belongs here, and we have actually had material from Josserand supporting this. None of the ancient descriptions fit this plant exactly, and it has probably been overlooked. It may possibly be *C. ephemerus* sensu Bisby, Buller and Dearness (**1929**).

7. COPRINUS CONGREGATUS Bull. ex. Fr.

Illustrations: Lange 1952, Figs. 2, 11.

Pileus 0.2–3.0 cm high, subellipsoid and rather narrow at first, expanding to conic campanulate with a flaring margin, subpubescent from cystidia, soon appearing almost glabrous, moist, sulcate-striate to the smooth disc, the disc and the backs of the striations "clay color" to "ochraceous tawny," marginal area "pinkish buff," finally dark gray over striate portion, sordid alutaceous over the disc; flesh very soft and fragile, entire cap deliquescing in humid weather, in dry weather only the gill edges deliquescing, no distinctive odor or taste; lamellae narrowly adnate, close (about 26 reach the stipe), narrow, whitish to "cartridge buff" when young, darkening to avellaneous and finally more sordid than "wood brown," edges white until deliquescence begins; spores ripening

from margin upwards or almost simultaneously; stipe white, 2–8 cm long, 0.6–3 mm thick, equal, hollow, pubescent from caulocystidia, strigose below, sometimes with a slight collar-like ridge and somewhat rooting.

Spores black in deposit, 9.0–14.7 × 5.4–7.6 μ, average 10.8–13.0 × 6.0–6.95 μ, moderately broad, $\frac{D}{d} = 1.75–1.95$, slightly flattened to almost terete, $d_1–d_2 = 0.0–0.5$, dark brown, fuscous to black in KOH, germpore distinct, slightly to very eccentric, always somewhat visible on abaxial side; basidia 18–30 × 9–11 μ, four-spored, di- or trimorphic; paraphyses subglobose, mostly 15–18 μ broad; pleurocystidia abundant, 70–120 × 20–50 μ, vesiculose, if more elongated the apices obtuse; cheilocystidia similar or somewhat shorter, subglobose, 30–70 × 20–30 μ, elongated-ventricose individuals rare to absent; gill trama thin, hyaline, hyaline to sordid brownish in KOH; cuticle of subglobose cells, 25–30 μ broad, oblong in the grooves where they are up to 65 μ long; pilocystidia 48–106(–150) × 7.5–17 × 4.5–12 μ, hyaline, subcylindric, slightly ventricose, apex obtuse.

Heterothallic, bipolar, the ability to form fruit bodies on diploid mycelia in some strains depending on a special gene, clamp connections absent on mycelium and in fruit body.

Habit, habitat and distribution: Solitary, gregarious or more often subfasciculate, on dung piles, on bits of manure in flower beds, hothouses, on soil mixed with kitchen refuse or on rotten straw, Sweden *, Denmark *, Germany, Iceland *, U.S.A.*

Observations: From its cultural characters this species is evidently identical with that studied by Brunswik (**1924**) under the name *C. ephemerus,* but it is hardly adequately described in recent literature. It is, however, in all probability the species Fries mentioned under *C. congregatus.* It shares the pale colors, grows subfasciculate, and Fries knew it from hothouses. In view of the inconsistent use of the name it might be preferable to rename the species, but this point of view could be taken for so many of the older species that one hesitates to accept it except for the most flagrant cases. If future work should show that *C. bisporus* is the next older name, and that *C. congregatus* sensu L. & S. is merely a four-spored form, the species epithet would be a most unfortunate choice.

8. COPRINUS BISPORUS J. Lange (1915).

Illustrations: J. Lange 1939, Plate 160, Fig. E; M. Lange 1952, Figs. 2, 11.

Pileus 0.5–2.5 cm high, subellipsoid and rather narrow at first, expanding to conic-campanulate, cystidiose, soon almost glabrous, sulcate-striate to the disc, pale ochraceous tawny to ivory white, the disc slightly darker ("clay color") or paler buff ("pinkish buff"), eventually fading to whitish, flesh thin, without distinctive odor and taste, deliquescing; lamellae narrow, crowded, adnexed to the top of the stipe or almost free, pallid when young, darkening from the margin upwards, margin white until deliquescing; stipe 2–10 × 0.1–0.3 cm, white-strigose below, rooting.

Spores black in deposit, 9.5–14.6 × 5.2–7.4 μ, $\frac{D}{d} = 1.8–1.95$, $d_1–d_2 = 0.15–0.55\ \mu$, dark brown, germpore distinctly eccentric and always somewhat visible in face view; basidia 18–33 × 6.5–7.5 μ, two-spored, di- to trimorphic; paraphyses subglobose-pyriform, about 16 μ broad; pleurocystidia rare, absent in most collections; cheilocystidia vesiculose, 20–60 × 15–30 μ, elongated-ventricose individuals scattered to absent; gill trama fibrillose, of very narrow hyphae and a few inflated subhymenial elements; pileus trama mostly of inflated elements, hyaline; cuticle of globose-subglobose cells 18–35 μ broad, oblong in the grooves and up to 60 × 25 μ; pilocystidia subcylindric, slightly inflated below, apex obtuse, 55–140 × 8.5–22 × 4–9.5 μ.

Homothallic, without clamp connections on the mycelium or in the fruit body.

Habit, habitat and distribution: On dung, often a mixture of dung and soil, subfasciculate, Denmark *, Sweden *, Greenland *, U.S.A.

Observations: This species is evidently that depicted by J. Lange (**1939**). It has the same growth form, the same pale colors and the same type of pilocystidia. It is closely related to *C. congregatus,* but differs in bispored basidia, which is a constant character in culture, smaller basidia, paler colors, and more distinct subfasciculate growth. In culture the two species are further distinguished by a pronounced brownish color of the mycelium of *C. congregatus.* Non-fruiting single spore mycelia of *C. bisporus* are intersterile with haplonts of *C. congregatus* (Lange **1952**).

9. COPRINUS STELLATUS Buller (1929).

Illustrations: Lange 1952, Figs. 3, 12.

Pileus 0.15–1.0 cm high, short cylindric-conic, the margin flaring and splitting at maturity, wrinkled-striate, becoming plicate, disc smooth, "ochraceous tawny" to "tawny olive," marginal area soon paler, becoming gray to black when spores mature, surface at first densely cystidiose from very small cystidia, glabrous in age; flesh very soft and delicate, no odor or taste, rapidly deliquescing; lamellae narrow and close, distant on very small specimens, free, pallid, becoming dark fuscous to black from the spores, edges white until deliquescence begins; stipe 1–5 × 0.05–0.25 cm, cylindric, densely cystidiose when young, base equal, sometimes rooting, sometimes slightly inflated, somewhat strigose, hollow, whitish or hyaline whitish, in some specimens brown (even as dark as bay brown) below.

Spores black in deposits, 7.9–13.3 × 4.4–6.4 μ, averages 8.8–12.1 × 4.9–6 μ, almost terete, moderately broad to rather narrow, $\frac{D}{d} = 1.75–2.05$, blackish brown in water mounts, black in KOH, much paler if not fully mature, germpore apical; basidia four-spored, 16–30 × 7.5–9(10) μ generally dimorphic, paraphyses almost globose, 17–24 μ broad, much narrowed in young stages; pleurocystidia abundant to scattered 70–130 × 15–40 μ; cheilocystidia rather variable, forming a broad band or more scattered, similar to pleurocystidia or some of them subglobose, always with some short, ventricose, acuminate ones between the vesiculose individuals, the fusoid-ventricose type seldom more than 30 μ long; gill trama mostly fibrillose, hyaline to pale brownish, pileus trama of short, somewhat inflated elements; cuticle of subglobose to globose cells 15–35 μ broad, not much longer in the furrows; pilocystidia very numerous and rather uniform in size and shape, hyaline or faintly brownish at base, 25–65 × 7–14 × 2.5–5 μ, acuminate.

Homothallic, clamp connections absent on mycelia and in fruit body.

Habit, habitat and distribution: Solitary, gregarious, or more rarely subfasciculate, on dung. Probably world-wide in distribution. We have seen specimens from Denmark *, England *, Iceland *, India, Sweden *, and U.S.A.*

Observations: *C. stellatus* is best characterized through the very abundant, small pilocystidia. Our material has shown some varia-

tion in spore size, and size and shape of the various cystidia, but none of these differences have been constant in culture. We have never seen the pleurocystidia as long as indicated by Buller, but for the rest, his description (in Bisby, Buller and Dearness **1929**) fits our plant excellently. It is highly probable that this is *Agaricus subtilis* Fries, but we deem it better not to revive this name.

Stirps *Pellucidus*

10. COPRINUS PELLUCIDUS Karsten (1883).

Pileus 0.15–0.7 cm high, obtuse to convex, plicate-folded, splitting as it expands and hence torn into segments, segments horizontal or slightly recurved before finally deliquescing, young buds rather distinctly striate, disc dark ("cinnamon brown"), rarely paler, striae on young bud flushed with this color, but whole marginal area soon paler before turning blackish gray from the ripening spores; flesh membranaceous and delicate, slightly bitterish, odor faintly rankish, often but tardily deliquescing; lamellae narrow, close, 16–35 reach the stipe, free, chocolate brown to blackish brown from the ripe spores, edges white; stipe whitish to pale brownish hyaline, 0.5–4 × 0.05–0.15 cm, filiform, densely pubescent when young, sometimes slightly inflated at base.

Spores 6.4–11.4 × 3.1–5.1 μ, averages 7–10.5 × 3.4–4.8 μ, terete, narrow $\frac{D}{d} = 2.0–2.15$, shortly cylindric to narrowly ovate in face view, similar or slightly reniform in side view, cocoa-color in water when fresh, rarely darker, chocolate color in KOH; germpore apical, apiculus very indistinct; basidia mostly 16–26 × 6–8 μ, four-spored, di- and trimorphic; paraphyses small, almost globose, 10–16 μ broad; pleurocystidia not seen; cheilocystidia balloon-shaped, up to 40 μ broad, sometimes mixed with a rather large number of acuminate cystidia, the latter type even dominant in some collections; gill trama fibrillose with some inflated elements, pileus trama similar, frequently rather dark brown; cuticle of subglobose to globose cells, 15–28 μ broad; pilocystidia variable in different specimens, and also sometimes on the same pileus, 20–125 × 5–11.8 × 2–10 μ, acuminate, hyaline, rarely brownish below.

Homothallic, clamp connections absent on mycelium and in fruit body.

Habit, habitat and distribution: Solitary (in culture sometimes subfasciculate) on dung, Finland, Norway *, Denmark *, U.S.A.*

Observations: The species is best characterized by the small narrow spores. Karsten (1883) gives the spore size as 7–9 × 4 μ, in good agreement with what is recorded above. We have seen the type collection, which is made up mostly of small bits of dung, and on which the fungus has become almost obliterated. It was, however, possible to find a few spores on a fragment of the stem, and they were typically like those described here, and we believe we are fully justified in accepting the Karstenian name for this species. *C. pellucidus* would seem to merit further study in order to determine whether the differences recorded in the size of spores and pilocystidia would establish the presence of additional taxa.

Stirps *Angulatus*

11. COPRINUS ANGULATUS Peck (1874).

Coprinus Boudierii Quélet.

Illustrations numerous: i.a. Konrad & Maublanc 1929, Plate 37, Fig. 11.

Pileus 0.4–2.5 cm high, pubescent when young, rather broad and obtuse, at first acorn-shaped, then ovoid to hemispheric, becoming expanded, the margin recurving, wrinkled striate-sulcate, dark tawny to "Mars brown" all over when young, sometimes with a faint purplish tint, marginal area fading to "ochraceous tawny" before it becomes lead-color to blackish from the spores; flesh thin and fragile, taste and odor not distinctive, strongly deliquescing in humid weather; lamellae close, becoming subdistant, moderately broad, pallid, becoming black, the edges white-fimbriate until deliquescing; stipe comparatively short, 1–5 × 0.1–0.3(–0.4) cm, pubescent, whitish, often watery brownish within.

Spores black in deposits, 7.3–11.6 × 4.5–8.1 μ, averages 8.05–10.25 × 5.85–6.5 μ, much flattened, d_1–d_2 = 0.85–2.5 μ, mitriform in face view, obliquely ovate-elliptic in side view, but varying much in shape inside this general pattern, dark brown to almost black, dark bistre brown to black in KOH, germpore apical, apiculus very small, often difficult to demonstrate; basidia 16–33(–39) × 7–10 μ, four-spored, trimorphic, all but the shortest ones pedicellate, ventricose, the longest slightly narrowed above midportion; paraphyses voluminous, 14–18 × 8–12 μ; pleurocystidia numerous to scattered, 55–125(–150) × (20–)40–70 μ; cheilocystidia dimorphic, on the covered part of the smaller gills similar to short

pleurocystidia, on the free gill-edges subglobose or ventricose bottle-shaped, the latter type being about 25–50 × 10–15 × 3.5–4 μ; gill trama of rather short, broad elements, subhyaline; pileus trama similar but tawny to cinnamon brown; pilocystidia 30–117 × 7–23 × 6.5–9.5 μ, somewhat inflated, mostly subacuminate (more obtuse types also seen), hyaline or brownish at the base, intergrading to more narrow, thick-walled sclerocystidia, the latter present in most specimens but in very variable number, in one collection measuring 60–82 × 6.5–9 × 3–3.5 μ, smaller in some collections.

Heterothallic, tetrapolar, clamp connections present on mycelium.

Habit, habitat and distribution: On burnt-over areas, attached to small fragments of charcoal, gregarious to solitary. We have seen material from U.S.A.*, Austria *, Denmark *, France *, Greenland *, Sweden *, and it is on record from additional countries.

Observations: The species is readily distinguished on the spore characters and on the peculiar habitat. Smith (**1948**) studied the type of Peck's species, making the status clear. If a velate species with the same type of spores really exists, as supposed by Josserand (**1944**), it should be described under a new name.

Stirps *Fallax*

12. **Coprinus fallax** sp. nov.

Statura *C. subpurpureo* et *C. plagioporo* similis. Sporae congestae atro-rubescentes, magn. 11–14 × 6–7.5 μ; basidia dimorpha, magn. 20–24 × 7–8 μ; pilocystidia magn. 20–30 × 100–120 μ.

Fibulae praesentes.

Ad terram caespitosus.

Typus in Dexter, Michigan sub numero 32096 ab A. H. Smith lectus, in Herb. Univ. Mich. depositus.

Pileus about 10 mm high before buttons expand, obtusely conic, expanding to convex or broadly conic, margin appressed to stipe at first, surface moist and hygrophanous, plicate-striate to the watery disc, soon splitting in the creases, color "walnut brown" to "Rood's brown" on disc, margin about "fawn color" (dark to pale vinaceous brown); flesh membranous and delicate; lamellae adnate to apex of stipe or barely touching it, subdistant, narrow, edges soon deliquescing and discolored, faces about concolorous with pileus; stipe 2–3.5 cm long, about 1 mm thick, equal,

hyaline, white or at base slightly discolored, surface faintly fibrillose.

Spores 11–14 × 6–7.5 μ, near "pecan brown" in water mounts of fresh spores, reddish in deposits, pallid in KOH mounts of dried material but soon changing to chocolate gray-brown, in side view slightly inequilateral, face view ovate, germpore more or less eccentric but very difficult to demonstrate on any but darkest spores; basidia dimorphic, 20–24 × 7–8 μ, hyaline, four-spored; paraphyses vesiculose, about 20 μ broad; pleurocystidia not seen; cheilocystidia (all collapsed); pileus trama with a cellular cuticle about 1 cell deep, the cells thin-walled and hyaline in KOH; pilocystidia numerous, (15–)20–30 × 100–120 μ (or shorter if neck is short), ventricose at base and with a long neck terminating in an obtuse apex, thin-walled, hyaline in water mounts; small hyaline sclerocystidia very rare to absent; clamp connections present on mycelium around base of stipe; caulocystidia similar to pilocystidia or more elongated.

Not cultured.

Habit, habitat and distribution: Cespitose on soil in a lumber yard, Dexter, Mich., May 24, 1949 (Smith—*32096*). It is known only from the type locality.

Observations: The vinaceous brown colors are distinctive in *Coprinus*, but other species with them are known. However, the color of the spore deposit is aberrant in the same degree as the spore color of the *Psathyrella sarcocephala* group is aberrant for that genus. The reaction of the spores in KOH reminds one of the reaction of the spores of some tropical species of *Psathyrella* in the same medium. In the character of deliquescing, in the characters of the hymenium, and in aspect the fungus clearly falls in *Setulosi* of *Coprinus*. The reaction of the spores in KOH definitely indicated relationships here rather than in *Bolbitius*.

Stirps *Plagioporus*

13. COPRINUS PLAGIOPORUS Romagnesi (1941).

Coprinus ephemerus f. *saturatus* J. Lange (1939)

Illustrations: Romagnesi (l.c.) Fig. 6; Lange 1952, Figs. 4, 13.

Pileus 0.5–1.2 cm high before expanding, buds rather broad, margin adpressed to stem, wrinkled-fisso-sulcate, plicato-striate when expanding, transversely rugulose in the grooves, at last

revolute, color rather variable, brownish, generally with a faint reddish hue, the disc being always darker than the sulcate part, sometimes as dark as "bone brown," but frequently paler, "wood brown" or even dull "vinaceous buff," the striae on the expanding cap slightly paler than the disc, paler (pale "ochraceous buff") between the striae, finally darkening as the spores ripen, densely cystidiose at first, often beaded with drops; flesh of cap delicate and thin, concolorous, taste slightly bitterish, odor none; gills moderately close, 28–36 reach the stipe, one to three tiers of lamellulae, rather narrow, broadest (about 2 mm) above but almost free, soon black from the spores maturing almost simultaneously over the whole gill; stipe 2–6 × 0.1–0.2 cm, slightly swollen below, hollow, whitish, more rarely flushed with the color of the cap at base, inside and outside, densely cystidiose; gills and (under humid conditions) the entire cap, deliquescing.

Spore print almost black, spores 9.1–14 × 5.4–7 μ, averages 10.9–12.05 × 6–6.4 μ, moderately broad to rather narrow, $\frac{D}{d} = 1.75–2.0$, slightly flattened, $d_1–d_2 = 0.3–0.6$ μ, oval in face view, elliptic with the adaxial side slightly flattened in side view, fuscous to almost black, with germpore moderately eccentric, always visible on the abaxial side; basidia 18–35 × 8.5–9.5 μ, four-spored tetramorphic; paraphyses numerous, subglobose-pearshaped, 15–20 × 23–33 μ; pleurocystidia not seen; cheilocystidia numerous, subglobose-balloon-shaped 18–45(–65)μ across, elongated-ventricose types (similar to pilocystidia) rare to scattered but observed in all specimens examined; gill trama sordid brown to hyaline; pileus trama paler or darker brownish, mostly of inflated elements; cuticle of globose to subglobose cells, mostly 25 μ broad, more oblong in the furrows, up to 90 × 50 μ; pilocystidia 43–103 × 9–20 × 5–12.5 μ, moderately inflated below, subcapitate, neck 4–10 μ broad; sclerocystidia rare to absent, when present similar to other pilocystidia but smaller, dark brown and very thick-walled.

Amphithallic, tetrapolar, clamp connections present on mycelium.

Habit, habitat and distribution: Solitary or subfasciculate, mostly on small sticks and the like, but also truly terrestrial, on black muck, etc., Denmark *, France, Sweden *, U.S.A.

Observations: This species is easily recognizable by the characters of the pilocystidia, which are very constant in size and shape. Except for the absence of pleurocystidia, our plants correspond well with the original description by Romagnesi (1941),

and so we have assigned them here. We have studied a spore print from the collection of J. Lange and found indications that *C. ephemerus* f. *saturatus* J. Lange covers this species (germpore moderately eccentric, spores dark, averaging 11.75 × 6.05 μ.).

14. COPRINUS SUBPURPUREUS Smith (1948).

Illustrations: Smith l.c. Fig. 25; Lange 1952, Figs. 4, 13.

Pileus about 1.5 cm high, cylindric to ovoid young and expanding to campanulate before the spores are shed, becoming nearly plane or even recurving when gills deliquesce, finely pruinose-pubescent when young, soon almost glabrous as cystidia collapse, folded-striate at first, broadly plicate-striate at maturity, disc dark vinaceous brown ("army brown," "natal brown" to "Benzo brown"), "light brownish drab" toward the paler ("light cinnamon drab") broad striate marginal area, in age "dark purple drab" over disc and dark gray to blackish over margin, vinaceous brown tinge often remaining over the backs of the striations; flesh very thin and fragile, delicate, no odor or taste; lamellae narrowly adnate, close, becoming subdistant, narrow, near "tilleul buff," darkening to black over all before the edges deliquesce; stipe 3–10 × 0.05–0.25 cm, equal, dull lilac umber young, rarely pallid, becoming paler in age, densely pruinose-pubescent but soon glabrescent, base white-strigose.

Spores black in deposit, 10.7–15.7 × 5.3–7.6 μ, averages 12.45–13.8 × 6.1–6.9 μ, narrow $\frac{D}{d} = 1.9–2.1$, flattened or not, $d_1–d_2 = 0.0–0.6$ μ, narrowly subovate to elliptic in face view, with a more or less distinct suprahilar depression in side view, blackish brown, germpore always very much visible on the abaxial side; basidia tetramorphic, four-spored, 18–30 × 8–10 μ; paraphyses hyaline, inflated; pleurocystidia none; cheilocystidia vesiculose, 20–30 μ broad, ventricose-fusoid ones rare to absent; gill trama thin and appearing cellular in sections, slightly to distinctly vinaceous brown in KOH; pileus trama thin and appearing to be a mixture of narrow filaments and enlarged cells, vinaceous fuscous in KOH, clamp connections present; cuticle of vesiculose cells; pilocystidia subcylindric, rarely much inflated below, apex obtuse, 53–120 (–150) × 8–20(–25) × 4–12.5 μ, somewhat variable in average size in different specimens; sclerocystidia not seen.

Amphithallic, tetrapolar, some specimens simply heterothallic tetrapolar, but the two types interfertile (Lange 1952), mycelium with numerous clamp connections.

Habit, habitat and distribution: Gregarious or solitary, in moist leaves or on wet black muck, U.S.A.* and Denmark *.

Observations: It has not been possible to find any morphological differences distinctive for the purely heterothallic strains. The species is closely related to *C. plagioporus,* but easily distinguishable by the pilocystidia and the richer purplish colors.

15. **Coprinus sclerocystidiosus** sp. nov.

Illustrations: Lange 1952, Figs. 4, 13.

Statura speciei praecedenti similis, pilei tamen diametro saepe minore, pallide cinnamomeus vel argillaceus.

Sporae fuscae vel pallide fuscae, magn. 10.5–15 × 5.3–7.5 μ, anguste ovali-ellipticae, poris excentricis ornatae; basidia tetramorpha; cheilocystidia globularia, diam. 13–20 μ; pilocystidia dimorpha, altera hyalina, magn. 42–100 × 7.5–15 × 3–7.5 μ, subobtusa, altera sclerocystidia incrustata, magn. 43–166 × 1.5–4.5 μ, acuta.

Fibulae praesentes. Heterothallus, tetrapolaris.

Solitarius vel. subfasciculatim ad terram silvaticam.

Typus 10.8.49 ad Lundeborg, Danmark sub numero 3073 a M. Lange lectus, in Mus. Bot. Haun. depositus.

Pileus 0.6–1.2 cm high, long remaining narrowly conic, then expanding and finally revolute, fisso-sulcate, faintly transversely rugulose in the grooves, pubescent from collapsing cystidia, pale to moderately dark brown, disc "ochraceous salmon" to "ochraceous tawny" or pale "cinnamon brown," striate part paler, soon darkening from the spores, disc remaining brown; flesh thin and fragile, deliquescing in humid weather; lamellulae close to subdistant, 34 reach the stipe, rather narrow, edge white; stipe 2–3 × 0.15–0.2 cm, white, faintly darkened at base which is somewhat strigose.

Spores 10.5–15 × 5.3–7.5 μ, averages (11.2–)12.35–13.5 × (6.2–)6.45–6.85 μ, narrowly ovate in face view, elliptic in side view, $\frac{D}{d} = 1.8–2.05$, slightly flattened, $d_1–d_2 = 0.2–0.5$ μ, color relatively pale fuscous in water mounts, in KOH not much darker, germpore large and usually almost fully visible on abaxial side; basidia 20–33 × 9–10 μ, four-spored, tetramorphic, paraphyses subglobose, 13–20 μ broad; pleurocystidia absent; cheilocystidia balloon-shaped, 13–46 μ broad (or in other specimens than the type mostly fusoid-ventricose and 27–35× 9–13 μ); pileus trama dull brownish, clamp connections seen on thin hyphae; cuticle of hyaline, subglobose cells, mostly 25 μ broad, sometimes

larger, more oblong in the furrows (80–90 μ long); pilocystidia very variable, hyaline ones 42–100 × 7.5–15 × 3–7.5 μ, bottle-shaped, neck narrowly cylindric, apex obtuse; sclerocystidia 43–166 × 5–13 × 1.5–4.5 μ, thick-walled, subhyaline to yellowish brown, slightly inflated below, pedicellate, tapering to a pointed apex, walls often somewhat irregularly wavy, in some specimens incrusted with hyaline, refractive material at the base, the two types of pilocystidia somewhat intergrading, in some specimens only sclerocystidia are seen, on other pilei they are rare.

Heterothallic, tetrapolar, clamp connections present on mycelium.

Habit, habitat and distribution: Scattered to gregarious or even subfasciculate, on wet, rich soil with scattered vegetation, along drives in woods, sometimes attached to small bids of wood, Denmark *, U.S.A. Type: Purreskov, Lundeborg, Denmark, 10–VIII–1949. M. Lange coll. *3073,* in the Univ. of Copenhagen Botanical Museum.

Observations: The description is drawn up on several Danish collections found to be interfertile in culture. We have two American collections from Idaho which possibly may belong here. They differ in having the cheilocystidia fusoid-ventricose and in addition the sclerocystidia differ slightly. These have not been studied in culture and their identification is considered tentative. When the sclerocystidia are abundant, as in most of the Danish collections, they characterize the species well.

16. **Coprinus callinus** sp. nov.

Illustrations: Lange 1952, Figs. 5, 14.

Statura speciei praecedentis similis, colore saturato, cinnamomeobadis; tarde deliquescens.

Sporae magn. 9.7–13.3 × 5.6 μ, subangustae, ovato-ellipticae, fusco-nigrescentes, poris subapicali-excentricis ornatae; basidia tetramorpha, magn. 23–35 × 7.5–9 μ; pleurocystidia nulla; cheilocystidia globularia, diam. 20–30 μ, nonnulla subfusiformia; pilocystidia acuminato-subobtusa, magn. 75–154 × 10–20 × 3.5–10 μ; sclerocystidia plus minus frequentia, illis similia.

Fibulae praesentes. Heterothallus, tetrapolaris.

In callibus silvaticis terricolus.

Typus 30.6.1950 ad Tiselholt, Danmark sub numero 3327 a M. Lange lectus, in Mus. Bot. Haun. depositus.

Pileus 0.5–1.7 cm high, narrowly conic when in bud, expanding but the edge hardly recurving, fisso-sulcate to the disc which is dark brown ("tawny olive" to dark "cinnamon brown" or even

pale "sepia") paler towards the margin; lamellae crowded, becoming subdistant, 30–40 reach the stipe, 1–2 tiers of lamellulae, rather narrow, broadest near the middle, narrowly adnate to almost free, pallid, blackening from the ripening spores, edge white; stipe 2.5 × 0.15 cm, sometimes longer, caulocystidia numerous, white, hyaline in smallest carpophores, base white or faintly brownish; lamellae tardily deliquescing, pileus deliquescing in very humid atmosphere only.

Spores 9.7–13.3 × 5.0–6.0 μ, averages 10.7–11.95 × 5.57–6.2 μ, moderately broad to rather narrow, $\frac{D}{d} = 1.75–2.0$, flattened slightly or terete $d_1–d_2 = 0.1–0.5$ μ, narrowly ovate-elliptic in face view, narrowly elliptic in side view, dark fuscous, germpore subapical to distinctly eccentric; basidia 23–35 × 7.5–9 μ, four-spored, tetramorphic; pleurocystidia absent; cheilocystidia subglobose to globose, 20–30 μ broad, generally with some few bottle-shaped cystidia intermingled (these similar to pilocystidia but shorter and more obtuse); gill trama of somewhat inflated, rather short elements, subhyaline; pileus trama pallid to vivid brownish; cuticle of subglobose cells, 20–25 μ broad, some more oblong seen in the grooves; pilocystidia long, slightly inflated below, acuminate, apex pointed to subobtuse, 75–154 × 10–20 × 3.5–10 μ, almost constantly mixed with a larger or smaller number of thick-walled brownish sclerocystidia similar to the other pilocystidia but slightly shorter, more pointed and brownish.

Heterothallic, tetrapolar, clamp connections seen on mycelium.

Habit, habitat and distribution: In moist places on the ground under sparse vegetation, more rarely on buried twigs and the like, solitary, gregarious or subfasciculate. Most collections are from along drives in the woods, Denmark *, U.S.A. Type: Tiselholt, Denmark, 30–VI–1950, in beech leaves, M. Lange coll. *3227*, in the Univ. of Copenhagen Botanical Museum.

Observations: The above description is based on several Danish collections found to be interfertile in culture. Several other Danish collections, forming a different interfertility group, can, however, not be distinguished on any other character. We have not cultured American material, but many American collections conform well with the above description and should be regarded as belonging in the same species, even if some deviate in minor characters such as slightly larger spores and more obtuse cystidia. The long, subacute pilocystidia and medium sized rather narrow

spores appear to be distinctive of *C. callinus*. Further studies, however, may show the necessity of recognizing taxa below the species level for some of the material we have encountered.

It is highly possible that this is *C. hiascens* in the sense of Romagnesi (1941), and it might also be *C. congregatus* of some authors and *C. sociatus* of other writers. The two first mentioned names are here used for other species, and the third name should presumably be discarded since the existing descriptions are very inadequate.

17. **Coprinus subimpatiens** sp. nov.

Illustrations: Lange 1952, Figs. 5, 14.

Statura et colore *C. callino* et *C. sclerocystidioso* valde affinis, sed plerumque major, sulcis latioribus, rugulis transversalibus manifestioribus ornatis.

Sporae magn. 9.3–13.5 × 5.7–7.1 μ, latae, poris subapicalibus-subexcentricis ornatae; pleurocystidia sacciformia, sparsa; cheilocystidia vesicularia, nonnulla ventricosa; pilocystidia magn. 62–110 × 9–17 × 5.5–8.5 μ, hyalina, fusiformi-ventricosa, apice subobtusa; sclerocystidia minora, saepe crebra.

Fibulae praesentes. Heterothallus, tetrapolaris.

In silvis terricola, subfascicularis vel gregarius..

Typus 22.8.1948 ad Ithaca, Michigan, U.S.A. sub numero 6273 a V. Potter lectus, in Herb. Univ. Mich. depositus.

Pileus 1.5–2.3 cm high, cylindric to ellipsoid at first, becoming obtusely conic with a flaring margin, pruinose when young, but soon naked, plicate-striate to the sordid "tawny" to "cinnamon brown" disc, paler "ochraceous tawny" to "ochraceous buff" or even whitish toward the margin, marginal area soon tinged lead-color (at least between the brownish ridges), transversely rugulose in the grooves, sometimes splitting; flesh very soft and thin, no odor or taste; lamellae close, about 28 reach the stipe, narrow, broadest above, narrowly adnate, white, becoming black, but tardily deliquescing, often crisped, edges long remaining whitish; stipe 2.5–10 × 0.15–0.3 cm thicker in young buds, equal, hollow, covered with caulocystidia, white to the base, loosely fibrillose at base, more appressed fibrillose above, glabrescent in age.

Spores black in deposit, 9.3–13.5 × 5.7–7.1 μ, broad $\frac{D}{d} = 1.6$–1.8, slightly flattened, d_1–$d_2 = 0.25$ μ, dark brown to almost black, coal black in KOH, germpore subcentral to distinctly eccentric; basidia tetramorphic, 18–32 × 8–10 μ; pleurocystidia scattered

(absent in some specimens), vesiculose to somewhat elongated with broadly rounded apices, 26–38 μ broad; cheilocystidia mostly vesiculose, up to 50 μ broad, bottle-shaped cystidia intermingled, the latter 85 × 18 × 7 μ, or smaller (mostly 25–40 × 10–13 × 5 μ); gill trama pale tawny in KOH near flesh of cap, hyaline elsewhere; pileus trama tawny in KOH, cuticle of subglobose cells, 25–35 μ broad, larger and more oblong in the grooves; pilocystidia 62–110 × 9–17 × 5.5–8.5 μ, hyaline, slightly brownish near the ventricose base, neck subcylindric, apex obtuse; sclerocystidia rare to abundant, some of them short and obtuse, others long, wavy (like thick-walled hyphae), dark brown.

Heterothallic, tetrapolar, clamp connections present on mycelium.

Habitat, habit and distribution: On mull or on more sandy soil, solitary, gregarious or subfasciculate, U.S.A.*, Denmark *, England *, Sweden *. Type: Ithaca, Mich., U.S.A., 8–22–48, leg. V. Potter (coll. *6273*), in Univ. of Michigan Herbarium.

Observations: The description covers American and Danish material found interfertile in culture and very uniform in morphological characters. Other Danish and Swedish collections and an English specimen have the same morphology, but form two other intersterility groups. They should be included in this species also. This species, which apparently has not been described previously, is close to *C. callinus* and also to *C. eurysporus*. It is distinguished on its broadly ovate spores and the medium sized obtuse cystidia.

Stirps *Eurysporus*

18. **Coprinus eurysporus** sp. nov.

Statura speciei praecedenti similis.

Sporae latiores, magn. 9–10.3 × 6.1–7.8 μ, rotundati-ovali-subglobulares; pleurocystidia fusiformi-ventricosa, sparsa, magn. 36–48 × 9.13 μ; cheilocystidia similia vel vesicularia; pilocystidia magn. 40–70 × 9–14 × 2.5–3.5 μ, acuminata, multa eorum sclerocystidia.

Humi in virgulis.

Typus 21.10.1947 ad Beaver Creek, Mt. Hood National Forest, sub numero 28033 ab A. H. Smith lectus, in Herb. Univ. Mich. depositus.

Pileus 1–2.5(–3) cm broad, subcylindric when young, becoming obtusely conic to convex, very finely pubescent at first, translucent-striate and finally becoming plicate-striate to the smooth disc, margin revolute when deliquescing, color "buckthorn brown"

to near "cinnamon brown," paler toward the margin, margin becoming gray; flesh very thin and delicate, no odor; lamellae crowded, narrow, adnate to stipe apex, edges pallid and crenulate at first, faces becoming dark over all before deliquescence begins; stipe 5–8(–9) × 0.25–0.5 cm, slightly enlarged downward, hollow, white, very fragile, splitting, minutely pruinose at first but soon glabrescent.

Spores 9–10.3 × 6.1–7.8 μ, compressed, d_1–d_2 = 1.0 μ, broadly wedge-shaped with rounded corners or almost circular in face view, in side view obscurely inequilateral to subelliptic, pore broad (2.5 μ), subapical, walls dark chocolate color to almost black in KOH; basidia four-spored, ventricose in midportion when sporulating, tetramorphic, 18–42 × 8–10 μ; pleurocystidia scattered, 36–48× 9–13 μ, fusoid-ventricose, hyaline, thin-walled, with narrow necks and acute apices; cheilocystidia a mixture of vesiculose cells and those resembling pleurocystidia; pileus trama tawny revived in KOH, cuticle of clavate to subglobose cells; pilocystidia scattered, fusoid-ventricose, hyaline, thin-walled, 40–70 × 9–14 × 2.5–3.5 μ, apices acute, some sclerocystidia also seen, similar to pilocystidia but thick-walled and brown.

Habit, habitat and distribution: Gregarious on sticks lying on the ground, Beaver Creek, Bear Springs Area, Mt. Hood National Forest, Oct. 21, 1947, Smith n. *28033* (type), in the Univ. of Mich. Herbarium.

Observations: The species is established on American material which was not obtained in culture. It is readily recognized by the acute, small cystidia and the short, broad spores. We have a single collection from Denmark which probably belongs here (Lange, coll. *3313*), but this differs in having sclerocystidia exclusively, and in slightly larger spores. Like other species in the group, it is tetrapolar, with clamp connections on the mycelium.

19. **Coprinus amphithallus** sp. nov.

Statura speciei praecedenti similior, sed minor.

Sporae magn. 11.2–15.6(–17) × 6.2–8.3 μ; basidia bispora, tetramorpha, magn. 24–40 × 9–10 μ; pleurocystidia nulla; cheilocystidia fusiformi-ventricosa, magn. 30–50 × 9–18 μ; pilocystidia 40–85 × 9–17 × 5–5.5 μ, ventricosa, apice obtusi-subacuta, hyalina, basi fuscidula.

Fibulae praesentes. Amphithallus.

In silvis terricolus.

Typus 30.6.1950 ad Tiselholt, Denmark sub numero 3222 a M. Lange lectus, in Mus. Bot. Haun. depositus.

Pileus 0.6–1.2 cm high, obtusely conic, expanding to campanulate, the margin revolute as deliquescence progresses, densely cystidiose at first, paler or darker brown "cinnamon brown," "Saccardo's umber" or "ochraceous tawny" over the disc and backs of striae, pallid or even whitish between striae, darkening as spores mature; lamellae narrow, close becoming subdistant, about 16 reach the stipe, adnate, pallid, becoming black before tardily deliquescing; stipe 3–5 × 0.08–0.1 cm, hyaline whitish, sometimes slightly brownish below, pruinose at first, soon glabrous.

Spores varying much in size on same cap, 11.2–15.6(–17) × 6.2–8.3 μ terete or somewhat flattened, d_1–d_2 = 0.6 μ, elliptic in side view, elliptic-ovate in face view, the larger ones often narrowed near apex so as to appear obscurely snoutlike, dark chocolate brown, germpore subapical to distinctly eccentric; basidia all two-spored, tetramorphic, 24–40 × 9–10 μ; paraphyses not distinctive; pleurocystidia absent; cheilocystidia fusoid-ventricose, small, 30–50 × 9–18 μ, apices subacute hyaline and thin-walled; pileus trama pale ochraceous in KOH, cuticle of vesiculose cells, pale yellowish or darker brownish (corresponding to color of cap), pileus trama ochraceous tawny, or darker; pilocystidia 40–85 × 9–17 × 5–5.5 μ, hyaline, except for the tawny to yellowish pedicels, ventricose, apex obtuse to subacute.

Amphithallic, tetrapolar, clamp connections present on mycelium.

Habit, habitat and distribution: On black mull or much in moist places, Denmark *, U.S.A. Type: Tiselholt, Denmark, June 30, 1950, M. Lange coll. *3222,* in the Univ. of Copenhagen Bot. Museum.

Observations: Only the Danish strain has been brought into culture, but the Danish and American material correspond in all morphological details except for slight differences in color. The species is recognized by the small pilocystidia with thickened basal walls. No four-spored *Coprinus* appears to be closely related.

Stirps *Hiascens*

20. COPRINUS HIASCENS (Bull. ex Fr.) Fr. sensu J. Lange.

Pileus 1.0–2.5 cm high, narrowly glandiform, then broadly convex, expanding rather late and hardly recurving, fisso-sulcate, transversally grooved, pallid, disc darker (pale "cinnamon brown"); flesh thin, without odor and taste, slowly but distinctly deliquescing in humid atmosphere; lamellae moderately crowded to subdistant, adnate to almost free, narrow, pallid, then almost

black from the spores; stipe 4–5 × 0.15–0.3 cm, hollow, white to the base, finely striate above, in young specimens with a few scattered fibrils, densely covered with caulocystidia, not very brittle, slightly enlarged below, hollow.

Spores 7.9–12.1 × 4.5–5.8 μ, averages 9.65–10.9 × 4.9–5.25 μ, hardly flattened, narrow, $\frac{D}{d} = 1.9–2.55$, narrowly ovate in face view, somewhat oblique in side view, apiculus very prominent, germpore large, almost apical, not visible in face view, brown; basidia four-spored, tetramorphic, very different in shape, the long ones much protruding and 36 × 6.5–7 μ, short ones 18 × 8.5–9 μ; paraphyses subglobose, pleurocystidia absent, cheilocystidia numerous, bottle-shaped, subacuminate, 26–75 × 12–18 × 4–7 μ; trama of gills scanty, of rather narrow and a few inflated elements; pileus trama of rather coarse elements, very pale cinnamon, cuticle of subglobose cells about 20 μ broad, longer in the grooves, pilocystidia 80–175 × 11.5–25 × 4–9 μ, very long, more or less inflated below, hyaline, some of them slightly brownish at base, no sclerocystidia seen.

Heterothallic, tetrapolar, clamp connections seen on mycelium.

Habit, habitat and distribution: In small clusters, rarely solitary, found both on rich and very light soil, Denmark.

Observations: The species is well characterized by the size and shape of the spores, resembling those of *C. disseminatus,* with which species it also has some likeness in basidial characters. In addition, the pilocystidia are quite characteristic. This is undoubtedly the species depicted by J. Lange (**1939**), and probably also the one described by Ricken (**1915**), except for spore measurements, which Ricken quotes from Karsten who presumably used the name for *C. plicatilis* or a species closely related to it. Romagnesi's species (**1941**) is presumably *C. callinus.*

Stirps *Disseminatus* [3]

21. COPRINUS DISSEMINATUS (Pers. ex Fr.) S. F. Gray (1821).

This species is so frequently described that a repetition of the macroscopic morphology is superfluous here (cfr. Buller **1913**,

[3] The American author is not in agreement with placing these non-deliquescing fungi in *Coprinus,* but they have been retained in this paper in order to put certain data on record. The conclusions recorded under this stirps are those of the Danish author.

1924 and Kuhner 1926). The following is a summary of the microscopic data.

Spores 7–9.4(–11) × 3.9–5.5 μ, flattened, d_1–d_2 = 0.25–0.6, rather broad $\frac{D}{d}$ = 1.6–1.9, broadly ovate in face view, obliquely elliptic in side view, apiculus rather conspicuous, germpore apical, relatively pale brown; basidia 20–55 × 5–10 μ, four-spored, distinctly tetramorphic, long type much protruding, very narrow; pleurocystidia none, cheilocystidia developed from the cap margin and about half way up the gill edges, rather scattered, large, obtuse, 60–115 × 16–21 × 7.5–10 μ; pilocystidia very large, 76–215 × 19–29 × 8–12.5 μ, inflated below, sometimes incrusted at the base, apex obtuse; sphaerocysts rather numerous, incrusted, 10–40 μ broad.

Heterothallic, bipolar, clamp connections present on mycelium.

Habit, habitat and distribution: Densely cespitose to gregarious, rarely subsolitary, on and around stumps. Almost cosmopolitan; we have seen material from several European countries, from U.S.A. and from India.

Observations: In growth form and stature and especially in the microscopic characters, this species is clearly a very close relative of *C. silvaticus*. There is a great likeness in the way the different types of cystidia are developed and in the rather outstanding type of basidia, also the spore shape is—in spite of differences in size and configuration—basically the same, and finally the type of sphaerocyst is identical. The two species are probably not very intimately related to the members of the subsection *Setulosi*, but more to the other fasciculate, semilignicolous species around *C. disseminatus*. This will leave no possibility for retaining the genus *Pseudocoprinus*.

22. **Coprinus subdisseminatus** M. Lange, sp. nov.

Pileus 0.3–0.8 cm altus, late convexus, deinde convexo-planus, sed non revolutus, distanter sulcatus, pallide cinnamomeus, velo debili, fibrillari primo indusiatus; stipes hyalino-albidus, disculo myceliari exiguo, membranaceo basi cinctus, non vel tardissime deliquescens.

Sporae magn. 8.8–12.7 × 5.5–6.5 μ, pallide fuscae; basidia plerumque trimorpha, tetraspora, magn. 19–33 × 9–10.5 μ; pleurocystidia nulla; cheilocystidia sacciformi-lageniformia, magn. 30–75 × 11–20 × 7–12 μ; pilocystidia magn. 59–121 × 10–22 × 7.5–15 μ, hyalina, subcylindrica, apice obtusa.

Fibulae praesentes.

In silvis humum uvidum et quisquilias inhabitans.

Typus 20.6.1950 ad Elsehoved Denmark sub numero 3216 a M. Lange lectus, in Mus. Bot. Haun. depositus.

Pileus 0.3–0.8 cm high, rather broadly convex in the bud, convex-flat when expanded, but not revolute, sulcate to the disc, the creases rather distant, more or less transversally grooved, pale brownish, "cinnamon buff" to "cinnamon," cystidiose; flesh very thin and fragile, pale, not deliquescing, odor and taste not distinctive; lamellae subdistant, 16–24 reach the stipe, adnate or slightly decurrent, pallid when young, dark "cinnamon brown" when spores ripen; stipe 1.3–2.5 × 0.05–0.15 cm, hyaline whitish to the base, slightly fibrillose, pubescent, on some very young specimens the fibrils from a rudimentary veil, attached to the substratum by a more or less developed whitish mycelial disc.

Spores 8.8–12.7 × 5.0–6.5 μ, averages 10.35–11.55 × 5.4–5.9 μ, mostly rather narrow, $\frac{D}{d} = 1.8–2.05$, germpore subapical to somewhat excentric, slightly visible on abaxial side on some spores, pale chocolate brown; basidia four-spored, mostly trimorphic, 19–33 × 9–10.5 μ pleurocystidia absent; cheilocystidia rather numerous to scattered, broad, obtuse, sack-shaped to bottle-shaped, 30–75 × 11–20 × 7–12 μ; trama of pileus and gills pale brownish, of moderately inflated to rather thin elements, cuticle of subglobose cells, 25–50 μ broad, larger and more oblong in the furrows, pilocystidia 59–121 × 10–22 × 7.5–15 μ, numerous to scattered, subcylindric, not much inflated below, apex obtuse, hyaline or almost so, no sclerocystidia seen.

Heterothallic, tetrapolar, clamp connections seen on the mycelium.

Habit, habitat and distribution: On wet, black mull or on sticks and other vegetable matter in humid localities, solitary or in small groups, but never seen subfasciculate; Denmark *. Type: Elsehoved, Denmark, June 20, 1950, M. Lange coll. *3216,* in the Univ. of Copenhagen Botanical Museum.

Observations: The species is distinguished primarily by its macroscopic characters, namely, the small, broad, pallid pileus. It is also well characterized by its cystidia. It has the appearance of *C. disseminatus* but its growth form is different, and the microscopical features are clearly distinct. We have an American collection, from Michigan, which may represent this species, but we have noted the cheilocystidia to be vesiculose.

23. COPRINUS IMPATIENS (Fr.) Quélet.

Pileus 1.5–3 cm high, broadly ovate at first, expanding to campanulate but edge not recurving, deeply grooved, even before opening, by alternating long and short impressions from the edge and about three quarters in, minutely cystidiose, pallid alutaceous, the disc slightly darker, fading to whitish when drying, becoming lead gray with a brownish tinge when spores mature, flesh thin and fragile, not deliquescing, lamellae subdistant, becoming fuscous when spores ripen; stipe 5–10 × 0.1–0.4 cm, somewhat enlarged towards the base which is white strigose, hollow, whitish, pubescent from cystidia.

Spores 8.5–11.1 × 5–6.1 μ, almost terete d_1–d_2 = 0.15–0.3 μ, rather broad, $\frac{D}{d}$ = 1.75–1.85, broadly ovate in face view, broadly elliptic in side view, pale chocolate color, germpore almost apical; basidia four-spored, pleurocystidia absent, cheilocystidia numerous, bottle-shaped to flask-shaped, generally subacute to acute, 20–62 × 10–16.5 × 3.5–5 μ; pilocystidia rather few, narrowly conical, acute to subacute, 50–86 × 10–16 × 3–4.5 μ, hyaline or slightly brownish below.

Heterothallic, tetrapolar, clamp connections present on mycelium.

Habit, habitat and distribution: Solitary, rarely subfasciculate, in deep foliage, Denmark *, France *, and the United States.

Observations: The species is one of the largest in the group, quite well distinguished by the broad grooves on the cap, the small, comparatively pale spores and the cystidia. *C. digitalis* in the concept of Romagnesi (1941) might be this species, but *C. digitalis* distributed by Lundell and Nannfeldt (No. *404*) most likely is a closely related but distinct species, recognized macroscopically by its more brownish gills, and microscopically by still smaller spores (7.85 × 4.7 μ) and also by the presence of some globose cheilocystidia between the bottle-shaped ones. The cultures deposited in Baarn under this name by Yen have been found interfertile with our material. The two last mentioned species are non-deliquescing, at least under ordinary conditions. Hence they belong in the genus *Pseudocoprinus* as generally defined at present. The American author would prefer to maintain this division while the European author finds the species too closely related to

members of the subsection Setulosi to make a generic separation possible (cp. Lange **1952**).

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BITANCOURTIA CASSYTHAE ON CASSYTHAE FILIFORMIS AND PROPOSED NOMENCLATORIAL CHANGES AMONG OTHER MYRIANGIALES[1]

M. J. Thirumalachar and Anna E. Jenkins[2]

(with 2 figures)

Near Bangalore, India, many plants of *Cassytha filiformis* are parasitized by a myriangiaceous fungus. The small dark stromata are abundant over the entire stem. Regeneration of new shoots may be prevented or plants killed outright. The ascomycete seems not to be characterized by any genera of the Myriangiales so far described.

Bitancourtia[3] gen. nov.

Stromata ascigera in pustulis conidiiferis mox evoluta, superficialia vel intra-epidermalia, erumpenti-superficialia, pulvinata vel pulvinato-applanata, sessilia, basi lata, tota matrici affixa; epithecium atro-fuligineum, ex cuticula et epidermidis parietibus superis ruptis et pseudoparenchymate obscuro formatum, friabile, particulis in superficiebus stromatis ascigeri persistentibus vel oram conspicuam interdum ruptam formantibus; stroma ascigerum brunnescens, griseolum, vel viridulum, et pulveraceum, intus prosenchymaticum, brunneo-flavidum, loculis numerosis, in stratis dispersis, ascum unicum ferentibus; hypothecium pseudoparenchymaticum, tenue, obscurum; asci globosi vel ellipsoidei; ascosporae elongato-obovoideae vel fusiformi-ellipticae, transverse 3-septatae, hyalinae; stromata conidiifera subcuticularia vel intra-epidermalia, palis conidiophororum obscuris; conidia minuta, 1-cellularia, hyalina vel lutescentia; microconidia adsunt.

Type: *Bitancourtia cassythae.*

[1] This study is a contribution under the Point 4 program, administered by the Technical Cooperation Administration, U. S. Department of State.

[2] Dr. Thirumalachar, Government Penicillin Factory, Poona, India; Dr. Jenkins, Service Foreign Agricultural Relations, U. S. Department of Agriculture, formerly of the Bureau of Plant Industry, Soils, and Agricultural Engineering.

[3] The name *Bitancourtia* is proposed in honor of A. A. Bitancourt, whose contributions to the study of the group have greatly enriched our knowledge.

Bitancourtia cassythae sp. nov. FIG. 1, A–G; 2

Maculae minutae, atrae nitidae, in caulibus omnibus dispersae, numerosae; hyphae inter- vel intra-cellulares, septatae, brunno-flavidae; stromata ascigera in pustulis conidiiferis mox evoluta, solitaria, gregaria, vel coalescentia, superficialia, vel intra-epidermalia, dein erumpenti-superficialia, pulvinata vel pulvinato-applanata, sessilia basi lata tota matrici affixa, 450–760 μ diam. $\times$ 130–160 μ crassa; epithecium atro-fuligineum, ex cuticula et epidermidis parietibus superis ruptis et pseudoparenchymate obscura formatum, usque ad 25 μ crassum, friabile, particulis in superficiebus stromatis ascigeris persistentibus vel oram conspicuam interdum ruptam formantibus; stroma ascigerum brunnescens, griseolum, vel viridulum, et interdum pulveraceum, intus prosenchymaticum brunneo-flavidum, loculis numerosis, in stratis dispersis, ascum unicum ferentibus; hypothecium pseudoparenchymaticum, tenue, obscurum; asci globosi vel ellipsoidei, 8-spori, 22–32 $\mu \times$ 11–19 μ; ascosporae elongato-obovoideae vel fusiformi-ellipticae, interdum asymmetricae vel curvatae, leves, transverse 3-septatae, hyalinae, 14–21 $\mu \times$ 4–5 μ; stromata conidiifera subcuticularia vel intra-epidermalia dein erumpentia, interdum ascomata cingentia, obscura, usque 180 μ diam. $\times$ 100 μ crasa; palis conidiophororum compactis; conidiophora obscura, oblonga, cylindrica, vel acuta, 1-pauciseptata, 5–26 $\mu \times$ 4–5 μ; conidia ellipsoidea, 1-cellularia, hyalina vel lutescentia, 3 $\times$ 5 μ; microconidia adsunt.[4]

In caulibus *Cassythae filiformis* L. (Lauraceae), Yashavantapur, Bangalore, South India, parasitica.

Yashavantapur, Bangalore, South India, Jan. 1, 1948, M. J. Narasimhan (Type). Specimens distributed as follows: Herb. Crypt. India Orient., New Delhi, India.; Herb. Royal Bot. Gard., Kew, England; No. *90598*, Mycol. Coll., U. S. Bur. of Plant Indus., Soils, and Agr. Engin., Beltsville, Md., No. *5319*, Herb. Secc. Fitop. Inst. Biol., São Paulo, Brazil, and No. *488*, Jenkins-Bitancourt, Myriangiales selecti exsiccati.

A large collection of *Cassytha filiformis*, Marshall Islands, 1951, F. R. Fosberg, was lent to us by the collector to examine for the possible presence of *Bitancourtia cassythae*. None was found.

In comparing the fungus on *Cassytha* with several myriangiaceous fungi previously described, *Myriangium pritzelianum* P. Henn. (2, p. 353) proved to be of interest. The fungus parasitizes leaves, stems and flowers of *Trymalium wichurae* Nees. (Rhamnaceae) in Australia, and the presence of numerous dark, pulvinate stromata especially on the stems suggested a similarity

[4] Thanks are expressed to Conrad V. Morton for translating the diagnoses of the new genus and species into Latin.

to *Bitancourtia cassythae*. A careful examination of the stromata, however, revealed that they are cinnamon color as described. This coloration is characteristic of *Uleomyces*. Comparison of the distribution of the asci with those of other species of *Myriangium* and of *Uleomyces* indicates that the fungus is more closely related to *Uleomyces* than *Myriangium*. We therefore propose to transfer it to *Uleomyces* as **U. pritzelianum** (P. Henn.) comb. nov. (FIG. 1, H, I).

The fungi concerned in the remainder of this paper were all discovered in South America.

The genus *Kusanoopsis* Stevens & Weedon (8, p. 199) was established for a myriangiaceous fungus on an undetermined dicotyledonous plant in British Guiana. The genus is so far monotypic. An examination of authentic material of the type species, *K. guianensis*, together with microtome sections prepared by Stevens and Weedon [5] and freehand sections made by the senior writer, demonstrates that *Kusanoopsis* is synonymous with *Uleomyces* P. Henn. (1, p. 107). The Lauraceae are known to be hosts for species of *Uleomyces*. A general comparison of the "unknown dicotyledonous host" of *K. guianensis* with herbarium specimens of Lauraceae shows that this may well belong to the same family.

It is proposed to reduce *Kusanoopsis* as a synonym of *Uleomyces* with the following synonomy:

Uleomyces guianensis (Stevens & Weedon) comb. nov.

Kusanoopsis guianensis Stevens & Weedon (1923)

On ? Lauraceae

Stevens and Weedon (8, p 201) described *Myriangina miconae* as inciting a small leaf spot on *Miconia* sp. in British Guiana. This fungus as described and illustrated by them is clearly of the genus *Elsinoë* Rac. emend. Jenkins & Bitanc. (4). Not only has this been noted by the writers, but it has been mentioned independently by both D. P. Limber and A. A. Bitancourt in correspondence with the junior writer. A reexamination of Stevens

[5] Stevens and Weedon's microtome slides mentioned in this paper are deposited with their specimens in the herbarium of the University of Illinois. They were kindly lent to the junior writer by Dr. Leland Shanor.

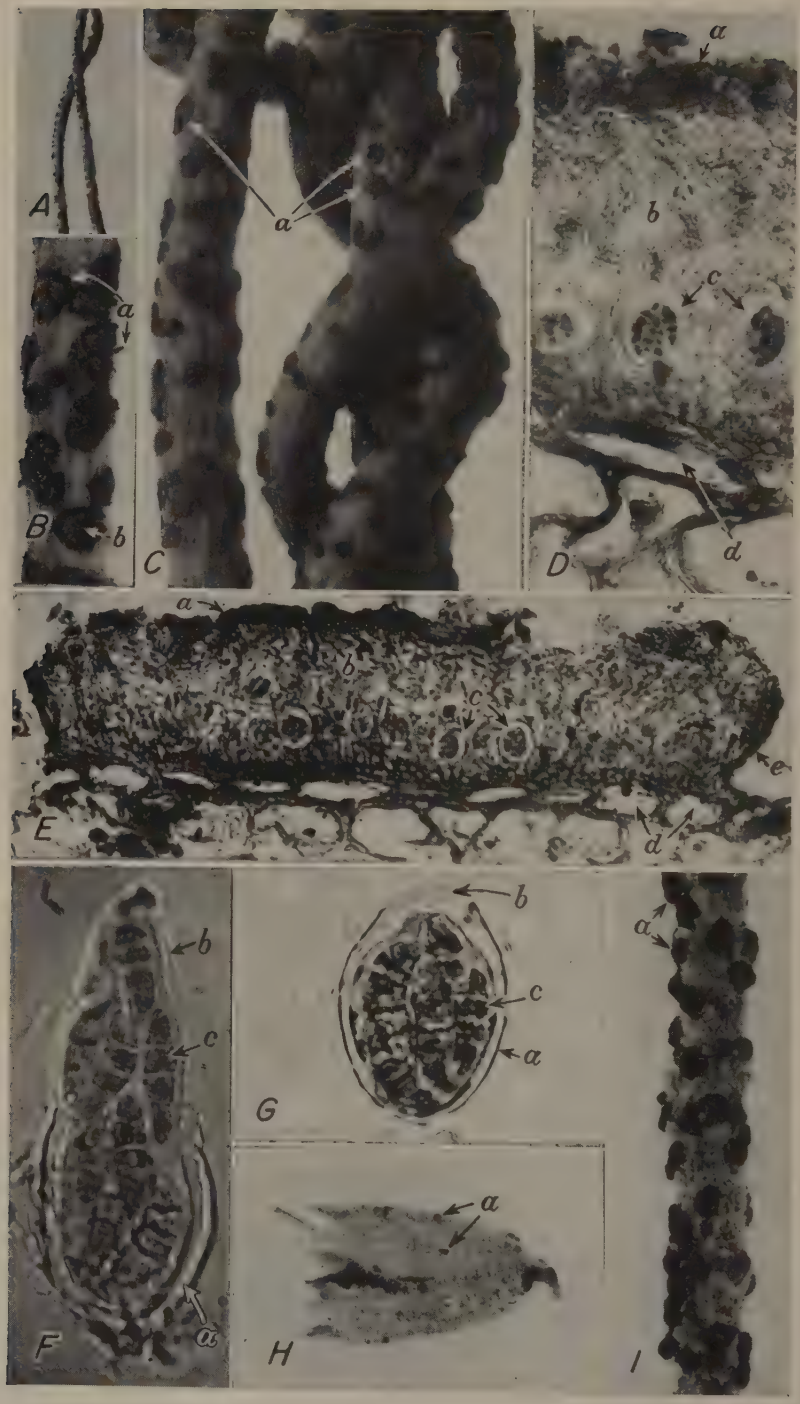

FIG. 1.

and Weedon's microtome section of this species also supports this conclusion. The following nomenclatorial change is proposed:

Elsinoë miconiae (Stevens & Weedon) comb. nov. Limber & Bitanc.

Myriangina miconiae Stevens & Weedon (1923)

The ascomycete on *Mimosa vellozina* described by Rehm (**5**, p. 169) as *Ascomycetella purpurascens* and transferred to *Myriangium* (*Uleomyces*) by Hennings (**3**, p. 56) was recognized more recently by Viégas (**10**, p. 59) as *Elsinoë purpurascens* (Rehm). On leaves, the fungus is restricted to the veins, forming the characteristic patterns described and illustrated by Viégas. Comparison of part of the type of *Chaconia berroana* Speg. (**6**, p. 29) on *M. flagellaris* sent to the junior writer by J. C. Lindquist with a specimen of *E. purpurascens* contributed previously by Viégas reveals that the two are identical. This myriangiale seems to conform most nearly to *Uleomyces*. The following combination is proposed:

Uleomyces purpurascens (Rehm) comb. nov.

Ascomycetella purpurascens Rehm (1901)

Myriangium (*Uleomyces*) *purpurascens* (Rehm) P. Henn. (1902)

Elsinoë purpurascens (Rehm) Viégas (1944)

Chaconia berroana Speg. (1912)

The genus *Whetzeliomyces* Viégas (**11**, p. 561) was erected to classify a myriangiaceous fungus on *Eupatorium* sp. in Brazil. The conidial stage of the fungus was described by Stevens (**7**, p. 371) on *Eupatorium* in British Guiana. In naming his ascomycete from Brazil, Viégas made the new combination *W. niger* (Stevens).

FIG. 1. *Bitancourtia cassythae* on stems of *Cassytha filiformis*. A–C. Ascomata, general appearance A, × 1; B. *a*, ruptured epithecium exposing the ascogenous stroma (*b*) beneath, × 10; C, × 9. D, E. Part of a stroma (D), × 280, and an entire stroma (E), × 160, in cross section. *a*, epithecium; *b*, fertile stroma; *c*, asci with ascospores; *d*, epidermis; E, *e*, darkened adhering cuticle. F, G, asci, *a*, outer inelastic wall, ruptured in F; *b*, inner elastic wall, expanded in F; *c*, ascospores; F, × 1250; G, × 1000. H, I. Stromata of *Uleomyces pritzelianum* on flower (H, *a*), × 6, and stem (I, *a*), × 12, of *Trymalium wichurae* (type). Photographs by Eugene Herling (A and C), R. L. Taylor (B, H and I) and M. L. Jaeger (D–G).

As restudied by the writers on the basis of the type specimen, this ascomycete is referable to the genus *Agostaea* Theiss. & Syd. (9, p. 359). A Latin diagnosis of the species is here provided under the name *A. nigra*.

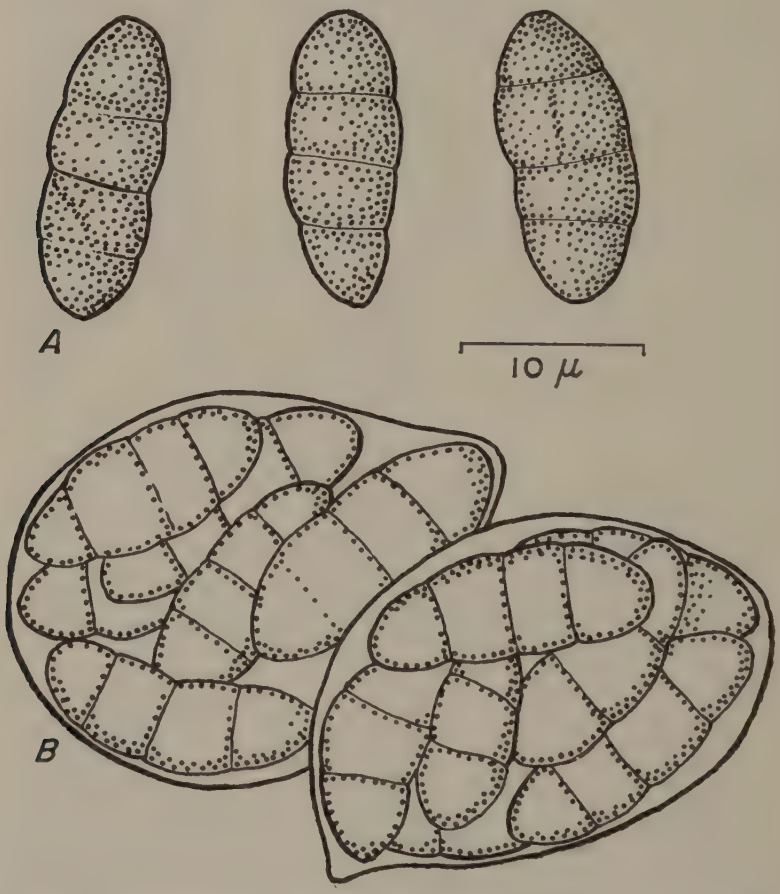

FIG. 2. Ascospores (A) and asci (B) of *Bitancourtia cassythae*. Drawings by D. P. Limber.

Agostaea nigra (Viégas) comb. nov.

Tuberculariа nigra Stevens (1901)

Whetzeliomyces niger (Stevens) Viégas (1945)

Stromata nigra, subepidermalia, erumpentia, hemispherica, lobulata, usque 400 μ in diam., 200 μ crassa, solitaria vel confluentia, e contextu pro-

senchymatico composita, olivaceo-viridia sed rubro-tincta; asci in strato singulo peripherici, e textura intertheci alia separati, subglobosi usque globoso-piriformes, bitunicati, octospori; ascosporae hyalinae sed humidae pigmentum stromatis absorbentes, muriformes, plerumque transverse 3-septatae, cellulis 1–4 verticaliter septatis, ad septa transversa subconstrictae, 11–16 × 5–6 μ.

Status conidicus: *Tuberculariа nigra* Stevens.

In foliis, caulibus et pedunculis *Eupatorii* sp., Brasilia.[6]

Type specimen: Mogi-Mirim, State of São Paulo, Brazil, Dec. 8, 1943, A. S. Lima and A. P. Viégas (No. *4333,* Herb. Inst. Agron., Campinas, Brazil; No. *20130,* U. S. Mycol. Coll.; No. *5395,* Herb. Inst. Biol).

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[6] This translation into Latin was kindly made by Edith K. Cash.

NOTES AND BRIEF ARTICLES

Systemic Infection in Cintractia junci[1]

In discussing the biology of the caricicolous species of *Cintractia*, Savile (Can. Jour. Bot. 30: 410–435. 1952) recently indicated that none appeared to be systemic, despite statements to the contrary; but that specimens suggested that the species attacking *Juncus* and *Luzula* appeared to be in a different category.

When *Cintractia junci* (Schw.) Trel. was found on *Juncus dudleyi* Wieg., near Ottawa, on 19 June 1952 (DAOM *28627*, Savile and Parmelee *3103*), this question was borne in mind. It was noted that culms were never lightly affected but were always completely blasted or completely normal. Infected culms were, on the average, shorter than normal culms and the inflorescence generally had abnormally short branches. It was further noted that infected culms tended to occur in groups, rather than being randomly scattered; and all infected culms were in an area about 3 feet in diameter, although the colony extended for several yards. A number of clumps were carefully dug, in order to preserve rhizome connections as completely as possible. One of the resulting clumps is shown in Fig. 1. All the culms arising from one part of the rhizome were healthy, and all those from the other part were infected.

Free-hand sections were made from various parts of infected culms and mounted in lactophenol with cotton blue. Extremely sparse mycelium could be seen in the xylem in the lower parts of the culm; but sections cut one-half inch below the base of the inflorescence showed plentiful mycelium both in and between the xylem elements. Such hyphae are absent from healthy culms. It is accordingly clear that the fungus is systemic and perennial.

It may be noted that the species of *Cintractia* attacking *Fimbristylis* and *Cyperus* appear always to be localized like those on

[1] Contribution No. 1270 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

FIG. 1. *Cintractia junci* on *Juncus dudleyi*, showing infected culms arising from part of rhizome in which mycelium was perennial.

Scirpus, Kobresia, Carex, and *Uncinia,* although infection is not confined to the ovaries. However, some, at least, of the species that attack *Rhynchospora* seem to be systemic. If all the species

attacking Juncaceae and all those on *Rhynochospora* prove to be systemic, then the type of habit would seem to have taxonomic significance.—D. B. O. SAVILE AND J. A. PARMELEE.

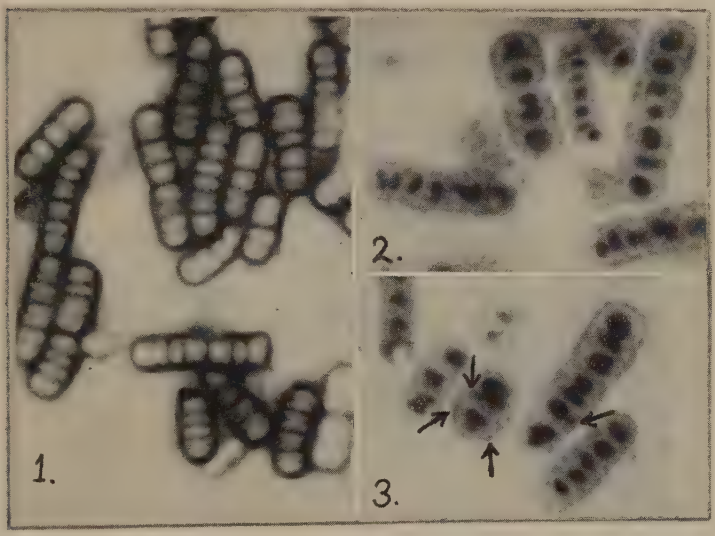
THE CYTOLOGY OF CARYOPHANON LATUM

In two recent papers DeLamater (**1951**, **1952**) has claimed to demonstrate true mitosis in the large bacterium *Caryophanon latum*. These are both illustrated with the same photomicrographs of material acid-hydrolysed, stained with thionin and dehydrated in freezing alcohol. It has been shown that this technique is not, as DeLamater claims, specific for the nuclear structures, but also stains the cytoplasmic septa of the multicellular bacteria which he (apparently unknowingly) employs for his demonstrations, and that, in addition, the method of dehydration produces gross distortion (Bisset, **1953a**). Indeed, in this laboratory it has been observed that exactly similar appearances to those figured by DeLamater can be obtained by the substitution of boiling for freezing reagents in his technique.

An excellent study of *Caryophanon* by Pringsheim and Robinow (**1947**) shows that the vegetative forms are composed of very small, disc-shaped cells, separated by cross-walls, as illustrated here (FIG. 1), and containing typical bacterial nucleoids (FIGS. 2, 3). DeLamater ignores this very striking and characteristic structure, which is clearly shown here in material stained by his method, but, instead of being subjected to his dehydration technique, sealed in water under a coverslip, and photographed immediately.

DeLamater's photomicrographs are, by comparison, very indistinct, and the most characteristic stage, the "metaphase," is represented in both papers by part only of one organism, of which the outline is barely discernible except as two vague marks which presumably represent the "centrioles." Similar stainable areas appear at or near the junctions of cell walls and cross-walls in many bacteria (Bisset, **1952**, **1953a**, **1953b**), and are indicated, in positions exactly corresponding with DeLamater's "centrioles," in FIG. 3. In FIG. 4 a drawing of this "metaphase" is compared

with a typical organism from FIG. 1 and one from FIG. 3, from which it can be seen that the claim to demonstrate a mitotic cycle with this as the sole example, in two papers, of the most characteristic stage, can only be regarded as rash. This, and all De-



FIGS. 1–3. The cytology of *Caryophanon latum.* All photomicrographs of material stained with aqueous dyes, unfixed and mounted in water; photographed immediately after being prepared. 1. Cell walls and cross-walls by phosphomolybdic acid and methyl green, × 2000; the cells are seen to be very small, discoid and numbering as many as six or seven in the very shortest organisms, up to twenty in the largest. 2, 3. Nuclear structures and basophilia of the cell envelopes by HCl-thionin, × 3000; the typical bacterial nucleoids are seen, and the granules at the junction of cell wall and cross-walls, indicated by arrows in 3, correspond to DeLamater's supposed "centrioles."

Lamater's photomicrographs, show appearances which are much more readily interpreted as the somewhat shrunken remains of typical bacterial nucleoids, as figured here and by Pringsheim and Robinow, than as true mitotic figures; especially as DeLamater's interpretation takes no cognizance of the remarkable cellular organization of *Caryophanon,* in which most, if not all, of his "mitotic figures" occupy the space of two, three or more of the relatively tiny, discoid cells of this strongly septate organism.

DeLamater claims also to have demonstrated mitosis in other types of bacteria. The validity of this claim may be examined by those who care to study the present writer's analysis of this evidence, much of which is based upon repeated republications of a single photomicrograph of a "metaphase spindle" (Bisset, **1952**, **1953b**). In every case, including that of micrococci, exactly as in the case of *Caryophanon*, the septate structure and mode of divi-

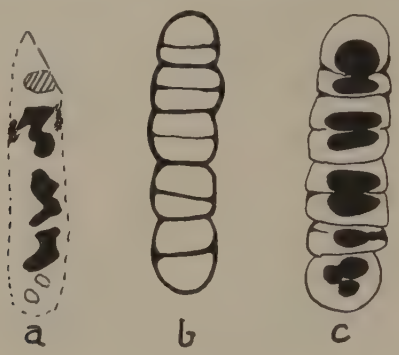


FIG. 4. Drawings comparing DeLamater's "metaphase spindle" (a) with appearance of organisms from FIGS. 1 and 2 in this paper (b and c). His interpretation of this organism, the sole representative of the stage in two papers (DeLamater **1951**, **1952**), is quite incompatible with the complex cellular structure of *Caryophanon*.

sion of these bacteria is ignored by DeLamater, and no evidence is offered in support of his crucial and readily disprovable assertions that his staining method is specific for nuclei and his dehydration technique preserves the structure of the cell unaltered.—K. A. BISSET, University of Birmingham.

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COMMENT ON ABOVE

A complete refutation of K. A. Bisset's contentions concerning this and other studies published by myself will be found in the International Review of Cytology, Volume 2, 1953.—E. D. DELAMATER.

AN UNUSUAL PSILOCYBE

A rather anomalous fungus has developed in a culture of cow dung from Montana which was sent to Dr. Alexander H. Smith. The normal fruiting bodies are those of an undetermined species of *Psilocybe* closely related to *P. coprophila*. The characteristic which attracted special attention to this plant was its production of an aberrant type of fruiting body. The microscopic characteristics of these structures are the same as those found in the normal fructification, but the carpophores are gastroid. The stipe is much reduced or absent and the pileus remains unexpanded. The hymenial layer is dispersed around the periphery of cavities formed by the folded gills. In gross form this type of fruiting body resembles a Gasteromycete more than an agaric. The gastroid fruiting bodies differ from the various types of carpophoroids which have been reported in that they produce viable spores. They have been produced repeatedly in agar culture along with normal fructifications and many intergrading types. Both normal and gastroid fruiting bodies have been obtained in culture from spores and from subcultures of diploid mycelium. No genetic basis for the inheritance of the gastroid form has been established.

While studying the gastroid form in culture, my attention was drawn to another anomalous form which apparently arose as a somatic mutation. This is an albino form which is completely devoid of color and has hyaline spores. It has been grown in culture through six generations from spores and is apparently quite stable. Both normal and gastroid fructifications are pro-

duced by the albino. They have been crossed with the normal form having colored spores and both albino and normal colored forms are obtained in the test crosses. Further experimentation is being carried on and it is hoped that a more detailed report of the behavior of these forms may be presented at some future time. A study of the albino form may be of interest to taxonomists, and particularly to those who have collected white forms whose microscopic characteristics show close affinities with the dark spored species.—KENT H. MCKNIGHT, University of Michigan.

A NEW SPECIES OF AMANITA

Amanita flavivolva sp. nov. Pileo convexo-plano, 6–7 cm lato, subflavo, disco griseo, grato; lamellis adnatis, confertis, 6 mm, albis; sporis 8 × 5–6 μ; stipite albo, flocculoso, 7–9 × 1–1.5 cm; annulo albo, amplo; volva flava, obliterata.

Pileus convex to expanded, scattered, 6–7 cm broad; surface pale-yellow with grayish disk, sparsely decorated with flat volval patches; margin even, entire, whitish; context white, unchanging, 4 mm thick, odorless, mild, becoming acrid; lamellae adnate, crowded, inserted, 6 mm wide, white, unchanging, fringed; spores broadly ovoid, 1-guttulae, about 8 × 5–6 μ; stipe tapering upward, white, unchanging, glabrous and ridged above the veil, flocculose-pulverulent below, 7–9 × 1–1.5 cm; annulus fixed 2 cm from apex, large and skirtlike, white with flavous edge; volva adnate to a small ovoid bulb, flavous, soon breaking into fragments remaining on the ground and cap.

Type collected by W. A. Murrill in an azalea bed under a laurel oak in Gainesville, Fla., July 16, 1950 (*F. 19598*). Near *A. gemmata* but having a yellow volva.—W. A. MURRILL.

THE PETERSHAM FORAY

The fourth annual foray (new series) of the Mycological Society of America was held August 18–20, 1949 at the Harvard Forest, Petersham, Massachusetts. The late Dr. W. Lawrence

White, then vice-president of the society, served as foray chairman. Dr. Hugh M. Raup, Director of the Harvard Forest, generously placed most of the facilities of this institution at our disposal. The foray was well attended, with 51 members and friends registering for the event. A few participants stayed in the town of Petersham. However, Shaler Hall, on the Forest grounds, provided living quarters for the majority of our group, and the large laboratory in the same building was made available for demonstrations, discussions, and specimen study.

The foray began at 9:30 A.M. on August 18th. Dr. Raup led the initial field trip and gave an informal lecture-demonstration of the various type localities and experimental plots in the forest. This served as a valuable orientation to the kinds of collecting areas available to the foray. The more avid collectors acquired a large number of specimens on this journey.

This foray became more marked by informal collecting than other forays have been. Throughout the afternoon of the 18th and all of the two following days small groups would vanish from Shaler Hall to visit different parts of the forest. The 2300 acres of the forest contained so many attractive collecting sites that the tendency for the foray to stick together in one large group (as was originally planned) did not persist very long. Each specialist turned to some area he had spotted that most interested him.

Dr. White scheduled a speaker for each evening the foray was in session. This departure from tradition was a striking success. Partially because of this the foray has been regarded by some of its participants as much a "talking foray" as a "collecting" one. The first lecture, presented on the evening of August 18th by Dr. V. M. Cutter, then of Yale, was entitled "*Sporobolomyces* as an organism for genetical and biochemical research." Dr. Cutter showed the group many cultures of *Sporobolomyces salmonicolor* Kluyver and van Niel, first demonstrating the range of variation in isolates from nature, then demonstrating the range of variation he had been able to induce through ultra-violet and x-ray irradiation. The variants selected for illustration were primarily those involving color strains. This material proved ample basis for a lively discussion centering around the following points: (1) The

possibility of using the mutation induction technique to recapitulate the course of phylogenetic evolution in a natural population; (2) The use of induced mutations prepared in the laboratory to predict the range of variation in a natural "species"; (3) The possibility of interpreting the survival values of any given mutation by studying the mutants in competition with the wild types in the field; (4) The possibility of using an organism such as *Sporobolomyces* as a tool for the study of experimental evolution.

Unfortunately the weather preceding the foray had been rather dry. Among the more interesting observations made, Dr. C. R. Orton noted that *Calyptospora columnaris* Kühn on *Vaccinium corymbosum* L. was abundant in one section of the forest and scattered in other areas. In one section where it was most abundant, there were several specimens of *Abies balsamea* (L.) Mill. in close contact with *Calyptospora*. Despite diligent search, in no case could rust in the *Abies* be found. Dr. Orton also reported abundant infection of *Uredinopsis* on *Osmunda* and again he could find no alternate stage on *Abies*. Dr. C. W. Dodge obtained material of *Elaphomyces asperulus* Vitt., a species seldom collected, though this area is not out of its expected range. Five species of myxomycetes and forty-three species of agarics were collected, which is of some interest considering the dry conditions that prevailed during and preceding the foray. Dr. C. J. Alexopoulos and Dr. C. T. Rogerson collected an interesting fungus on spiders, later identified by Dr. E. B. Mains as *Gibellula pleiopus* (Vuill.) Mains (cf. *Mycologia* **42**: 318–319. 1950).

The lecture on Tuesday evening (August 19th), given by Dr. James W. Sinden of Pennsylvania State College, was entitled: "Recent developments in the mushroom growing industry." A large amount of experimental work, done primarily by Dr. Sinden and his colleagues, was described in some detail and the foray members were given a vivid picture of the manner in which some of this research had given rise to technical advances in the industry. Dr. Sinden pointed out that in modern plants the mushroom industry has become almost completely mechanized since the introduction of the tray system in 1934. The compost is turned mechanically and filled into trays mechanically. It is then pas-

Annual Foray, 1949, Harvard Forest, Petersham, Massachusetts

Left to Right: Top Row: White, Beneke, Fergus, Rogers, Walters, Mrs. Walters, Pady, Bergman, Chable, Howard, Smith, Mrs. Overholts, Miss Haff, Mrs. Dodge, Johnson. Middle Row: Porter, Mrs. Alexopoulos, Miss Weintraub, Mrs. Snell, Conners, Cutter, Snell, Kneebone, Reese, Rogerson, Miss Kuhnen. Botton Row: Alexopoulos, Burlingham, Scott, Sproston, Mrs. Sproston, Dodge, Henry, Orton, Raup.

teurized in a specially insulated and equipped room. After mycelium has started here the trays are covered with an inch of soil mechanically and removed to the growing room where optimum environmental conditions for production of the crop can be maintained. Dr. Sinden described the development of a synthetic compost which competes with horse manure in price, but this has not been widely adopted because of the necessity of obtaining and combining the necessary ingredients. Composting practice has been changed to reduce the great loss in dry weight formerly experienced, resulting in considerable conservation of the ever dwindling supply of horse manure. Spawn for use in planting the beds is now largely made on grain, as this medium, being granular, is easier to handle than the solid manure spawn formerly sold. It is easier to make and also provides a richer medium, giving the mushroom a faster start in the bed.

Your foray chairman requested the writer to prepare this report for the society. The report was prepared but its submission has been delayed because for some time no adequate group picture of the foray could be located for reproduction with the report. These difficulties were recently solved by Mr. Maurice B. Walters, who kindly provided the negative from which the accompanying figure has been reproduced.

The reporter is also indebted to the individual members indicated below for reports from which the attached list of species has been compiled. Members reporting are individually identified as follows: [A]= Dr. C. J. Alexopoulos; [B]= Dr. E. S. Beneke; [H]= Dr. L. K. Henry, Jr. (Specimens deposited in the Carnegie Musum); [O]= Mrs. Marie Overholts (Specimens in Carnegie Museum); [P]= Dr. C. L. Fergus (Specimens collected by J. W. Sinden, E. T. Reese, L. R. Kneebone and/or C. L. Fergus and deposited in the herbarium of Pennsylvania State College); [R] = Dr. C. T. Rogerson; and [S]= Dr. Walter S. Snell.

LIST OF SPECIES REPORTED

Myxomycetes: *Diderma testaceum* (Schrad.) Pers. [A & B]; *Didymium Iridis* (Ditmar) Fr. On *Sphagnum* [R3185]; *Physarum globuliferum* (Bull.) Pers. [A & B]; *Stemonitis fusca* Rost. [A & B]; *Stemonitis Smithii* Macbr. [A & B].

Phycomycetes: *Endogone pisiformis* Link. On *Sphagnum* [P; R3180; S]; *Sporodinia grandis* Link. On decaying agaric [R3183].

Ascomycetes: *Ceratostomella ulmi* (Schwarz) Buisman. On *Ulmus Americana* [P]; *Chlorosplenium aeruginosum* (Oeder.) deN. [H]; *Diatrype stigma* (Hoff.) Fr. On dead hardwood [P]; *Dibotryon morbosum* (Schw.) Th. & Syd. On *Prunus* sp. [P]; *Helvella elastica* Bull. [H]; *Endothia parasitica* (Murr.) And. & And. On *Castanea dentata* [P]; *Geoglossum glabrum* (Pers.) Fr. In *Sphagnum* [R3178]; *Guignardia Bidwellii* (Ell.) V & R. On *Vitis* sp. [P]; *Hypocrea gelatinosa* (Tode) Fr. On decaying wood [P]; *Hypocrea rufa* (Pers.) Fr. On *Diatrype stigma* [R3189]; *Hypomyces lactifluorum* (Schw.) Tul. On *Lactarius* [P]. On agaric [R3190; R3191]; *Hypomyces viridis* (A. & S) Berk. & Br. On the hymenium of *Russula* sp. [R3188]; *Hypomyces violacea* (Schm.) Tul. On a myxomycete in *Sphagnum* [R3187]; *Hypoxylon coccineum* (Bull.) Fr. On dead *Fagus grandifolia* [R3175]; *Lasiosphaeria newfieldiana* Ellis & Ev. On fallen leaves in damp depression [R3177]; *Leotia lubrica* (Scop.) Pers. [P]. On mossy log [R3182]; *Leotia viscosa* Fr. On rotten log [R3181]; *Nectria cinnabarina* (Tode) Fr. On *Acer saccharum* [P]; *Nectria episphaeria* (Tode) Fr. On *Diatrype stigma* [R3193]; *Ophionectria cerea* (Berk. & Curt.) Ellis & Ev. On *Diatrype stigma* [R3194]; *Patella scutellata* (L.) Morgan [P]; *Phaeobulgaria inquinans* (Fr.) Nannfeldt [H]. On *Quercus borealis* [P]; *Trichoglossum hirsutum* (Pers.) Boud. [H].

Basidiomycetes (except for Polyporaceae, Boletaceae and Agaricaceae—see below): *Calocera cornea* (Batsch.) Fr. On *Tsuga canadensis* [P]; *Calyptospora columnaris* Kühn. On *Vaccinium corymbosum* L. [Orton]; *Clavaria cinerea* (Bull.) Fr. In coniferous woods [R3174]; *Clavaria cristata* (Holmsk.) Pers. [H]; *Clavaria fusiformis* Sowerby [H]; *Clavaria pyxidata* Pers. [O det. H]; *Dacrymyces palmatus* (Schw.) Bres. [S]; *Elaphomyces asperulus* Vitt. [Dodge]; *Hymenochaete agglutinans* Ellis [P]; *Irpex cinnamomeus* Fr. On dead hardwood [P]; *Scleroderma aurantium* (Vaill.) Pers. [H]; *Sebacina incrustans* (Pers.) Tul. Incrusting herbaceous plant [P]; *Septobasidium pinicola* Snell [S]; *Steccherinum septentrionale* (Fr.) Banker [H]; *Stereum fasciatum* Schw. On *Betula* [P]; *Stereum gausapatum* Fr. [P]; *Stereum rameale* Schw. [P; H]; *Thelephora anthocephala* (Bull.) Fr. On ground, coniferous woods [R3172]; *Thelephora multipartita* (Schw.) Fr. On ground, mixed woods [R3173]; *Thelephora palmata* Schw. [H]; *Uredinopsis mirabilis* (Peck) Magn. II. On *Onoclea sensibilis* [R3171]; *Uredinopsis osmundae* Magn. II. On *Osmunda* [Orton].

Polyporaceae: *Daedalea confragosa* (Bolt.) Fr. On *Betula* [P; H]; *Daedalea Quercina* (L.) Fr. On *Quercus* [P]; *Daedalea unicolor* (Bull.) Fr. [H]; *Favolus canadensis* Klot. [H]; *Fomes applanatus* (Pers.) Wallr. [H]. On *Acer saccharum* [P]; *Fomes fomentarius* (L.) Gill. [H]. On *Betula* [P]; *Fomes subroseus* (Weir) Overh. [H]; *Lenzites saepiaria* (Wulf.) Fr. On wood [P]; *Polyporus abietinus* (Dicks.) Fr. [H]; *Polyporus adustus* (Willd.) Fr. [H]; *Polyporus albellus* Peck. On *Betula* [P]; *Polyporus betulinus* (Bull.) Fr. [H]. On *Betula* [P]; *Polyporus borealis* Fr. [H]. On *Tsuga canadensis* [P]; *Polyporus elegans* (Bull.) Fr. On dead hardwood [P]; *Polyporus gilvus* (Schw.) Fr. On dead

hardwood [P]; *Polyporus nidulans* Fr. On *Betula* [P]; *Polyporus pargamenus* Fr. [H]. On dead hardwood [P]; *Polyporus semipileatus* Pk. [H]; *Polyporus semisupinus* Berk. & Curt. [H]; *Polyporus sulphureus* (Bull.) Fr. [H]; *Polyporus Tsugae* (Murr.) Overh. [H]; *Polyporus Tulipiferus* (Schw.) Overh. [H]; *Polyporus versicolor* (L.) Fr. [H]. On dead hardwood [P].

Boletaceae: *Boletinus pictus* (Pk.) Pk. [H; S]; *Boletus miniato-olivaceus* Frost [S]; *Boletus pallidus* Frost [S]; *Leccinum scabrum* (Bull.) S. F. Gray [S; O det. H]; *Leccinum subglabripes* (Pk.) Singer [S; H]; *Porphyrellus gracilis* (Pk.) Singer [S]; *Pulveroboletus retipes* (Berk. & Curt.) Singer [S; H]; *Suillus granulatus* (L.) Kunze [H]; *Suillus granulatus* (L.) Kunze ssp. *Snellii* Singer [S]; *Suillus subaureus* (Pk.) Snell [S]; *Tylopilus eximius* (Pk.) Singer [S].

Agaricaceae: *Amanita flavoconia* Atk. [H]; *Amanita mappa* Fr. form "A" [O det. H]; *Amanita rubescens* Fr. [H]; *Amanita virosa* Fr. [H]; *Amanitopsis vaginata* (Bull.) Roze var. *fulva* (Schaeff.) Sacc. [H; P]; *Amanitopsis vaginata* (Bull.) Roze var. *livida* Pk. [H]; *Cantharellus aurantiacus* Fr. [H; P]; *Cantharellus cinnabarinus* Schw. [H]; *Clitocybe ectypoides* Pk. [H]; *Clitocybe laccata* (Scop.) Fr. [H]; *Clitocybe laccata* (Scop.) Fr. var. *striatula* Pk. [H]; *Clitocybe odora* (Bull.) Fr. [O det. H]; *Collybia platyphylla* Fr. [H]; *Collybia radicata* (Relh.) Fr. var. *furfuracea* Pk. [H]; *Cortinarius armillatus* Fr. [H]; *Craterellus cornucopoides* (L.) Fr. [P]. On moss covered log [R3179]; *Crepidotus malachius* Berk. & Curt. [H]; *Entoloma strictus* Pk. [H]; *Galera sphagnorum* Sacc. [H]; *Hygrophorus Cantharellus* Fr. [H]; *Hygrophorus lactus* Fr. [H]; *Hygrophorus marginatus* Pk. [H]; *Hygrophorus miniatus* Fr. [H]; *Inocybe repanda* (Bull.) Quél. [H]; *Lactarius camphoratus* (Bull.) Fr. [O det. H]; *Lactarius deceptivus* Pk. [H; S]; *Lactarius griseus* Pk. [O det. H]; *Lactarius lignyotus* Fr. [H]; *Lactarius subdulcis* Pk. [H]; *Lactarius volemus* Fr. [H]; *Nyctalis asterophora* Fr. On blackened agaric [R3192]; *Panus strigosus* Berk. & Curt. [H]; *Panus stypticus* (Bull.) Fr. On wood [P]; *Paxillus atrotomentosus* Fr. [H]; *Paxillus panuoides* Fr. [S]; *Pholiota Johnsoniana* (Pk.) Atk. [H]; *Pholiota squarrosoides* Pk. [H]; *Russula alutacea* Fr. [H]; *Russula compacta* Frost & Pk. [S]; *Russula decolorans* Fr. [H]; *Russula foetens* (Pers.) Fr. [H]; *Russula fragilis* Fr. [H]; *Russula Mariae* Pk. [O det. H]; *Russula nigricans* (Bull.) Fr. [S]; *Russula xerampelina* Fr. [H].

Fungi Imperfecti: *Amblyosporium botrytis* Fresen. On *Lactarius* [P]; *Gibellula pleiopus* (Vuill.) Mains. On spider [A & R3186]; *Isaria arachnophila* Ditm. On spider in web [P]; *Septoria Lobeliae* Pk. On leaves of *Lobelia cardinalis* [R3170].

The thanks of the society were conveyed by all members present to Dr. W. Lawrence White, foray charman, and to Dr. and Mrs. Hugh M. Raup for planning this foray and for the excellent supervision and assistance they rendered to all members of our group.
—G. T. JOHNSON, Department of Botany and Bacteriology, University of Arkansas, Fayetteville, Arkansas.

REVIEWS

Erwin Frink Smith. A Story of American Plant Pathology, by Andrew Denny Rodgers III. x + 675 pp., 4 portraits. American Philosophical Society, Philadelphia 1952. Price, $5.00.

This biography of one of the first American plant pathologists should be of interest to anyone working in pathology or related fields. It presents not only a detailed account of the life and ideas of Smith himself, but also includes quite detailed commentaries concerning many other prominent men in various professions with whom he came in contact. His work with bacterial plant diseases is discussed in great detail, and much emphasis is placed upon his interest in human disease problems.

The author had access to Smith's diaries and papers and hence shows an intimate insight into the character of the man. This unfortunately emphasizes Smith's isolation from other plant pathologists, since many of the letters and notes are from workers in the medical field. Consequently, the book does not give a competent picture of plant pathology at the time at which Smith was active.

The frequent interruptions to discuss another person at great length and the extreme attention to detail may be disconcerting to some readers. It is, however, a record of an era in the development of plant pathology that should be familiar to present-day workers.—Lois H. Tiffany.

An Introduction to the Taxonomy and Nomenclature of the Fungi, by G. R. Bisby. Second edition, vii + 143 pp. Commonwealth Mycological Institute, Kew, Surrey, 1953. Price 10 shillings ($1.40).

The first edition of this book achieved a success which cannot be regarded as remarkable considering the surprising amount of information compressed into a little over a hundred pages. The

exhaustion of the first edition and the substantial number of changes in the rules made at Stockholm have made a revision desirable and welcome.

The first part, Introduction and ten chapters on taxonomy, has been reprinted with little change from the first edition. The second part, Notes on Classification, is entirely new and includes a chapter on Species and Other Concepts, and one each on the five major groups: Myxothallophyta, Phycomycetes, Ascomycetes, Basidiomycetes and Fungi Imperfecti. Part III, on Nomenclature, has the same chapter headings as Part II of the earlier edition, with the new Code, as revised at Stockholm, substituted for the earlier Rules, and the very useful examples of application of the articles and recommendations drawn from the fungi added where this seems desirable.

There is no work to be had for a comparable price which will be more useful to a young mycologist and there are few experienced students of the group who cannot read this admirable discussion with profit.

INDEX OF PLANT DISEASES IN THE UNITED STATES. PART V. PINACEAE—ZYGOPHYLLACEAE. INDEX. Compiled by Freeman Weiss and Muriel J. O'Brien. Plant Disease Survey, Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Dept. of Agriculture. pp. 809–1263 (multigraphed). Washington. 1953.

With the completion of the last part of this monumental undertaking there is now available to mycologists a complete and up-to-date host index to parasitic fungi. For the convenience of plant pathologists, for whom, of course, the work is primarily intended bacterial, virus and nematode diseases and various others are included. The index is in three parts: to Latin host names, to common names and to others.

It is the intention of the Survey to issue Supplements "as often as occasion demands and time permits." With that promise, a vary great need has been met and mycologists will find the Index constantly useful.

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HISTOPLASMA DUBOISII AND AFRICAN HISTOPLASMOSIS

R. VANBREUSEGHEM [1]

(WITH 6 FIGURES)

In a recent paper, A. Dubois, P. G. Janssens and P. Brutsaert (**1952**) have described the first case of histoplasmosis in the Belgian Congo. The mycological description of the parasite was made by myself (**1952**) and I considered on the basis of the parasitic as well as of the saprophytic characters that I could make a new species of this fungus, *Histoplasma duboisii,* previously undescribed as new. As a matter of fact, a careful study of the literature, as well as personal correspondence with my friend J. T. Duncan, has revealed that the same parasite has probably been encountered several times and always in cases of African origin. Lecéne (cited by Brumpt, **1936**), A Catanei and P. Kervran (**1945**), P. Kervran and R. Aretas (**1947**), Friess and Delvoye (**1947**), J. T. Duncan (**1947**), and, finally Dubois, Janssens and Brutsaert (**1952**), have been, as far as I can judge, the sole authors who have described human cases of histoplasmosis in which the parasitic cells were very large (*duboisii* form) as contrasted with the small parasitic cells commonly encountered in classical histoplasmosis (*capsulatum* form). In the second edition of the

[1] This investigation was made possible by financial assistance from the Institut pour la Recherche Scientifique en Afrique Centrale.

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Précis de Mycologie (Langeron and Vanbreuseghem), I have already given a brief discussion of histoplasmosis of African origin, and it seems that there is some reason to speak of an African histoplasmosis caused by *Histoplasma duboisii* as distinct from the classical histoplasmosis caused by *Histoplasma capsulatum*. This problem has been recently discussed by A. Dubois and R. Vanbreuseghem (**1952**).

It is probably not out of place to give some additional and more complete information on this interesting parasite.

The patient who was the source of the new strain was a white male of Belgian origin who had been for the last six years engaged in agricultural work in the central part of Belgian Congo. Some six months before his return to Belgium, swelling of the lymph nodes had been noticed in the neck and he had suspected that they were the first symptoms of African sleeping sickness. Later on, swelling of lymph nodes developed in the axilla. Puncture of the lymph nodes and histological examination of a node taken by biopsy in the cervical region revealed the presence of large and numerous parasitic cells which, afterwards, were also found in the sputum. These cells were for the most part included in large histiocytes or free in the surrounding medium. A search for the cells in the sternal marrow remained entirely negative.

MORPHOLOGICAL FEATURES OF THE PARASITIC CELLS

The parasitic cells as they were encountered in the lymph nodes of the patient are ovoid cells 13.5 $\mu \times 10\ \mu$. They possess a thick double-contoured capsule, 1–2 μ, and contain a very large amount of fat in the form of a unique, perfectly round globule which fills the cell almost completely, or of smaller globules, sometimes very numerous, of a spore-like appearance. Their real nature is, nevertheless, easy to demonstrate by mixing one drop of pus with one drop of Cotton Blue-Sudan in Chlorallactophenol, which gives to the fat a mahogany color, or by covering a dried smear of the pus with a 2 percent solution of osmic acid, which colors the fat grey. The Cotton Blue-Sudan—which, like the osmic acid, must act a long time (20 to 24 hours) to reveal the fatty nature of the inclusions—demonstrates at the same time some protoplasm in the

cell. When, exceptionally, the cell is devoid of fat, this appears as fine blue granulations dispersed in the cell or, when the cell contains a larger fatty globule, orients itself at its two poles.

The thick capsule is most often quite smooth; sometimes it is thicker than usual and, in such cases, it seems to be made of stratified layers, like a starch grain. Under careful observation these layers may show some cleavage and in these particularly

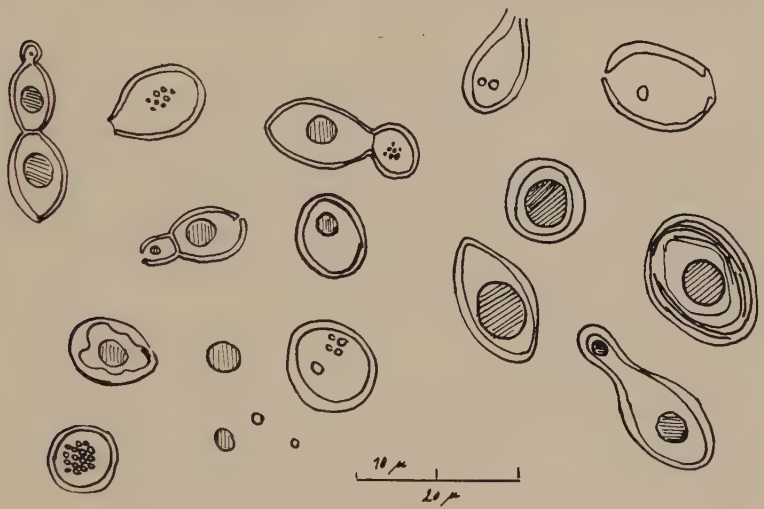


FIG. 1. *Duboisii* forms of *Histoplasma duboisii* from the lymph nodes of the patient, July, 1952.

thick capsules one may notice occasionally a notch in their most superficial layers.

Beside these typical cells one finds rather numerous budding cells. The bud begins on the smallest of the two poles of the ovoid cell. A thin membrane encloses it at the very beginning; this membrane seems to be the internal layer of the capsule of the mother cell and sometimes chains made of 4 or 5 successive cells may be found.

Gradually, in proportion as it grows, the membrane of the daughter cell becomes thicker and transforms itself into a capsule identical with the one of the mother cell; it follows that one may find in the smear, chains of 3 to 5 cells of the same size and of the

same morphological features. It is then impossible to decide which of the two terminal cells has been the source of the others.

In the sputum, the parasitic cells were rare and their size smaller than those of the lymphnodes (about 10 μ in their greatest diameter). The fat in these cells was generally more dispersed than in the cells of nodal origin.

In none of the examinations made of the pus from the lymph nodes, or of the sputum, was the classical small parasitic form of *Histoplasma capsulatum* encountered.

CULTURES OF HISTOPLASMA DUBOISII

It was quite easy to cultivate the fungus from the pus of the lymph nodes. On Sabouraud agar (glucose 2 percent) or on blood agar (sheep or rabbit blood), I secured a fair development at 25° C, maybe a little faster on the blood agar than on the Sabouraud agar. It takes, nevertheless, about 10 to 15 days to have a well developed colony. Although the richness of the pus in parasitic cells was enormous, the number of colonies obtained per slant did not exceed a good tenth. From the first cultures it was easy to obtain subcultures on the same medium or on glucose broth, soil agar with or without sugar, and even on Smith's synthetic medium.

MACROSCOPIC MORPHOLOGY OF THE COLONIES

1. *On Sabouraud agar (glucose 2 percent).*

At their very beginning the colonies look just like ground glass. In a few days they become white and downy, and grow very regularly, producing above the slant surface a disk 2–3 mm in thickness. The subcultures do not assume the ground glass appearance and are, at first, white and downy. With aging, the colonies become powdery and of a brownish or ochraceous color: this particular appearance is the result of the development of very numerous chlamydospores. Examined from the bottom, through the agar, the colonies, lightly immersed in the medium, show a chestnut color.

The centrum of the colonies is sometimes gently umbilicate, sometimes elevated above the rest of the colony.

There is a tendency to pleomorphic degeneration, variable from one culture to another, and it is necessary to make subcultures every 3 or 4 weeks to maintain the strain in normal condition.

2. *On blood agar.*

On blood agar, the colonies remain very superficial and develop like a thin membrane; later, they become brown and powdery but they never acquire the thickness they have on Sabouraud agar.

3. *On soil medium.*

On Sabouraud agar enriched with soil and on "conservation" medium enriched with soil, the growth of *Histoplasma duboisii* is very much the same as on plain Sabouraud. On soil agar—i.e., on a medium containing as sole nutrients: soil, agar and water—the development of the fungus is scanty but evident.

4. *In liquid medium.*

The growth starts in the deep layers of the liquid; the colonies are composed of downy mycelium, spherical or, when they adhere to the wall hemispherical. At a later stage, the superficial layer of the medium is invaded by the fungus; the colonies look like wax and are honeycombed, but at the margin they are powdery. In Smith's medium they are white, swollen and powdery.

MICROSCOPIC MORPHOLOGY OF HISTOPLASM DUBOISII

Most of our observations have been made on slide cultures made following the technic of Rivalier and Seydel. The observation is easy and the development of spores is abundant, perhaps more abundant than in slants. Every culture was grown at 25° C on Sabouraud agar. The inoculum was either the pus obtained from the patient or cultures obtained from this pus.

1. *Colonies developed directly from the pus.*

Most of the time, the result of inoculation on slides was a complete failure. In some cases we get a very slight growth of hyphae

but we have not been able to observe the development of those hyphae from the parasitic cells. These had completely disappeared after a week's incubation: sterile and most often coremioid hyphae had taken their place. It seems impossible to conclude from those observations anything other than the difficulty of obtaining primocultures on slides.

2. *Subcultures on slides.*

We started either with first cultures, 30 days old, or with subcultures 15 days old: the morphology of the colonies was the same in either case. The observation of slide cultures of different ages made it possible to obtain information on the structure of the colonies and among their constituents I have been able to recognize the following elements:

a. *The vegetative and aerial mycelium.*

The vegetative hyphae, about 2.5 μ in breadth, septate, with colorless walls, grow towards the periphery; they are slightly wavy. The length between two septa is 30 μ. Secondary vegetative hyphae arise from the principal hypha generally just behind a septum, but sometimes at a point as far as half-way between two septa. The angle made by the principal and the secondary hypha is wide and may be equal to a right angle. At the point where the secondary hyphae arise on the principal one, the latter quite often changes its direction of growth so as to correct the general direction of growth with its former line of growth. This aspect creates a false figure of dichotomy. Aerial hyphae arise on the principal hypha at a right angle; at their starting point, they make a cork screw-like movement. The space separating two septa is shorter in aerial hyphae than in vegetative hyphae; when they produce microconidia, the segments are no more than a few microns in length but when they produce macroconidia they are much larger (from 15 to 30 μ). The origin of the aerial hyphae is behind the septum just as in the case of the secondary vegetative hyphae; when the last ones have sufficiently grown they also may produce aerial hyphae.

b. *The spores: Microconidia and Macroconidia.*

Microconidia grow on both sides of the hyphae—generally aerial hyphae. They are pedunculate, round and their diameter is about 2.5 μ. The hyphae from which they arise are divided into short sections and the microconidia are borne, close to one another, or on opposite sides in rows of 20 to 30. When they are very numerous, the hyphae from which they arise is empty and is hardly visible; its figure is suggested by the position of the microconidia. The peduncle is very narrow (maximum, 1 μ) and no more than 2 μ in length. Exceptionally, one may see a microconidium arising from another one and I have found a chain of 3 microconidia attached one to the other.

Macroconidia are of two types: they are smooth, or covered with very irregular spines (echinulated chlamydospores). When ripe, their diameter is about 13 μ; they are prefectly round, and a slender sporophore, 1 to 2 μ in diameter, short, or up to 30 μ or more in length, connects them with the mother hyphae. The smooth macroconidia have a thick double-contoured membrane. The external wall is smooth and regular. The internal wall is smooth and regular but sometimes depressed on the contents of the macroconidia. The contents, sometimes clearly visible, are mainly composed of drops of fat. The chlamydospores or echinulated macroconidia have also a thick double-contoured membrane, but whereas the smooth and regular internal wall is scarcely visible, the outer one is covered with very irregular spinous overgrowths (2 μ long and 2 μ in diameter).

Are the microconidia related to the macroconidia, and the smooth macroconidia to the echinulated macroconidia? It is difficult to answer this question. I do not think that the microconidia are just small, unripened macroconidia: their position on the hyphae is very different. The sporophore of the microconidia is always very short which, on the contrary, is not the rule for the macroconidia. I do not think, either, that the smooth macroconidia are a mere stage in the formation of echinulate macroconidia. Actually, one can see in the cultures small echinulate macroconidia as well as large and mature ones. Besides, in old cultures, smooth macroconidia may be much more numerous than

echinulated chlamydospores. Furthermore, in my slide cultures, I have always seen the echinulated macroconidia before I could find any smooth macroconidia.

EXPERIMENTAL INOCULATION OF THE HUMAN PUS IN ANIMALS

White mice have been inoculated with pus diluted in physiological water (1 vol. pus/1 vol. water) intracerebrally (0.05 ml) and in the peritoneal cavity (0.25 ml). No symptoms developed. Guinea pigs have been inoculated with the pus (same dilution) in the peritoneal cavity (0.5 ml) or in the testis (0.2 ml). None of the animals became infected.[2]

It seems that it is impossible to transmit *Histoplasma duboisii* to the animal starting from the parasitic human cells. On the contrary, as may easily be ascertained, the inoculation of the saprophytic form is easy, at least to the guinea pig, when one makes use of the testicular route.

EXPERIMENTAL INOCULATION OF THE CULTURES OF HISTOPLASMA DUBOISII

The inoculum was made from 15 day old subcultures whose surface was gently rubbed with a glass rod in 3 ml physiological water per slant. The emulsion was composed mainly of spores.

Inoculation of white mice.

Six white mice were injected, in the peritoneal cavity, with 0.5 ml of the infecting emulsion. Two of them were killed 8 days later but nothing special could be noticed. Two others were killed 15 days after the inoculation. In one of the two, on the mesentery, small whitish nodules 1–2 mm in diameter were found. They contained very numerous parasites of the *capsulatum* form. The two last animals died but the autopsy could not be performed.

From this experience we may conclude that it is possible to inoculate *H. duboisii* in white mice but as far as we know the

[2] After this paper has been submitted, this inoculation was found possible. See R. Vanbreusegem, A. Dubois, P. Brutsaert and P. G. Janssens: Transmissibilité au cobaye d'Histoplasma duboisii à partir de la forme humaine. Proc. Soc. Belg. Méd. Trop. **33**: 171–176. 1953.

evolution of the parasite does not go farther than the *capsulatum* form stage. It is evident that other experiments must be undertaken before it is concluded that the *duboisii* form does not appear in the white mice.

Inoculation of rabbits.

Two rabbits received by the subcutaneous way 3 ml of the same emulsion used for the white mice. The next day a slight swelling was noticeable at the point of injection but a week later no more symptoms were observed. Nearly 7 months later one of the two

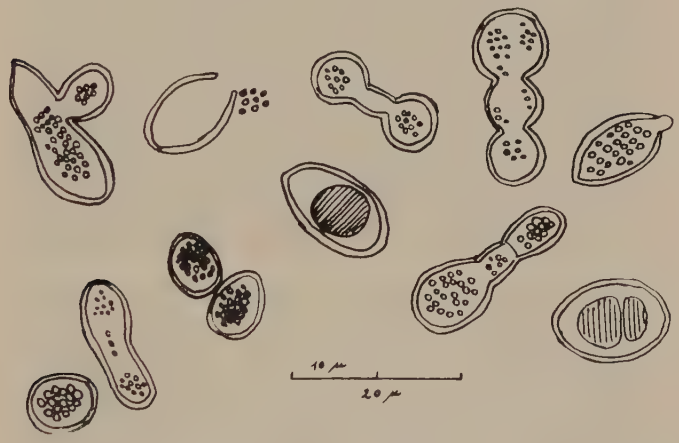
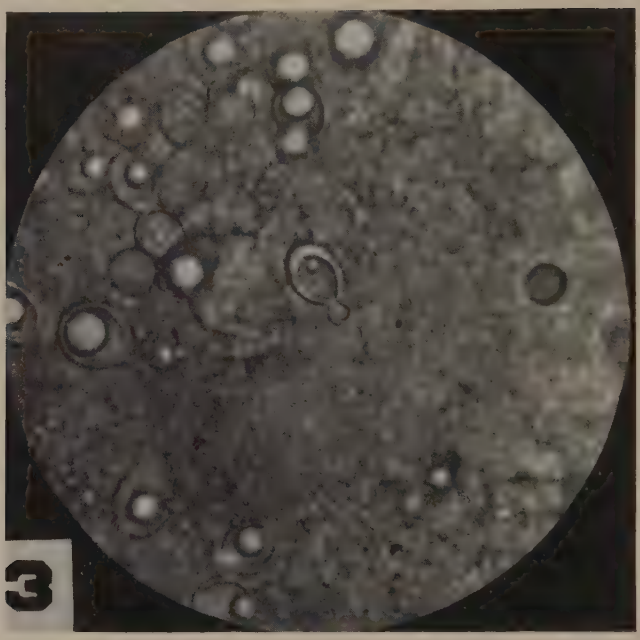


FIG. 2. *Duboisii* forms of *Histoplasma duboisii* from the testis of a guinea pig four months after inoculation.

animals died. An autopsy was performed. A small quantity of a clear effusion was found in the pericardic cavity and a yellowish tumor in one of the two lungs. Culture of this tumor did not give any result. The second animal was still alive and apparently healthy 8 months after the inoculation.

Inoculation of guinea pigs.

I made use of the testicular route and injected in the right testis 0.5 ml of the same emulsion utilized for mice and rabbits. Two guinea pigs were inoculated and about every week the testis was punctured with a fine needle. Eight days after inoculation, the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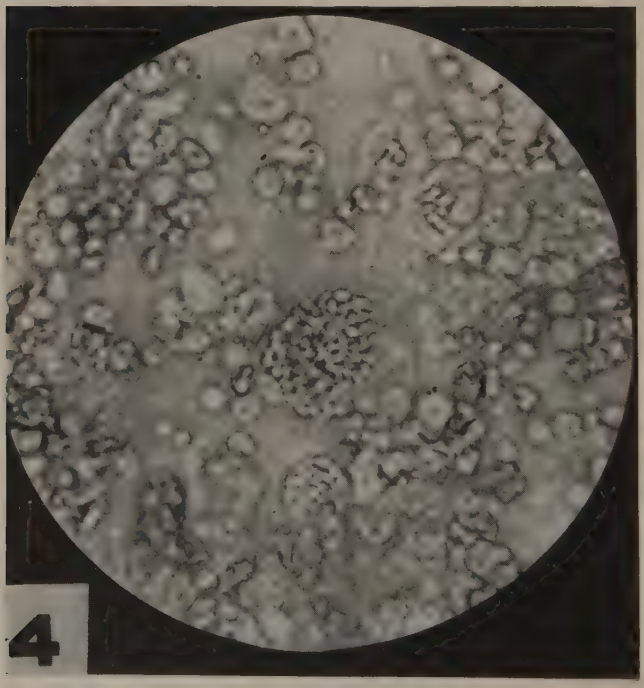
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puncture was ineffective in one of the animals but in the other one showed some echinulated chlamydospores. Eight days later, i.e., 15 days after inoculation, numerous parasitic cells of the *capsulatum* form were found in the testis of both animals. Three weeks after inoculation the parasitic cells were always very numerous but a good many were of larger size than the typical parasitic cells of *Histoplasma capsulatum* and recall the morphological figure of the parasitic cells of *H. duboisii* as found in the patient.

Three weeks after the inoculation, cultures were obtained from the product of the puncture of the testis of both animals. Contrary to what happened with the human pus, the cultures from the testis of the guinea pigs were very abundant. It is to be noted that at this stage the *capsulatum* forms were still much more abundant than the *duboisii* forms.

About two months after the inoculation one of the two guinea pigs died. Necropsy revealed a slight hemorrhagic peritoneal exudate without any parasites. Parasites were found only in the inoculated testis, mainly in the *duboisii* form, much more rarely in the *capsulatum* form. The cause of death was not evident.

Four months after inoculation the second guinea pig was found to be severely ill. The eyes were injected; ulcers 2 mm × 4 mm were developed on the right inferior palpebra. Small yellowish tumors were discovered on both sides of the palpebral conjunctiva; numerous *duboisii* forms were found in the conjunctival sacs. In the neck as well as in the inguinal regions big lymph nodes had developed. The two testes had been replaced by large irregular masses as big as a half nut. An autopsy was performed (chloroform). The two testes had been pushed back in the abdominal cavity; they were enlarged as well as the epididymis. The scrotal masses found on the living animal were not the testes but lymph nodes. A large lymph node had developed on the back side of the right eye. Peritoneum, liver, lungs, spleen, right kidney seemed normal. The left kidney was enlarged. *Duboisii* forms

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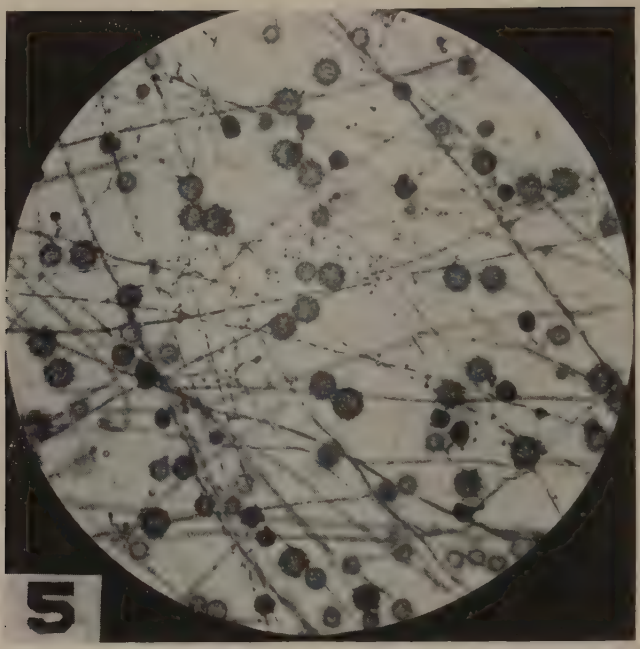
FIGS. 3, 4. 3. *Duboisii* forms in the lymph node of the patient. Stained with Cotton Blue-lactophenol, × 880. 4. *Capsulatum* forms packed in a large histocyte in the testis of a guinea pig 15 days after inoculation with *Histoplasma duboisii*, × 500.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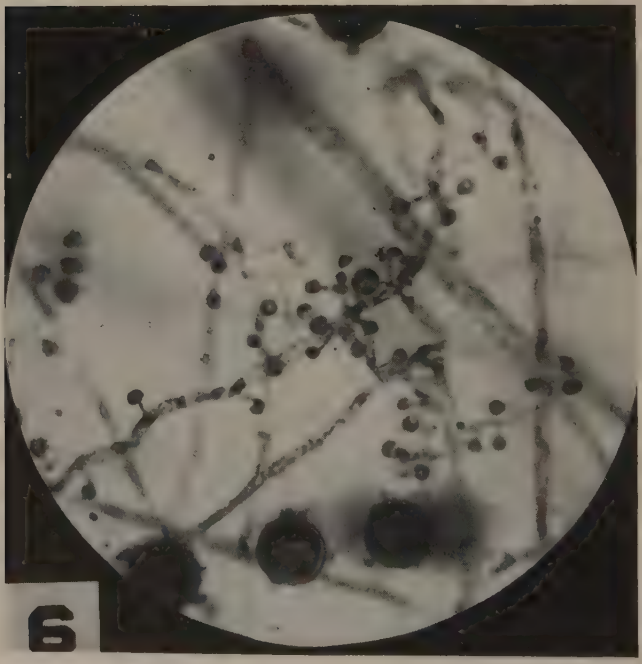
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were found in the two testes, liver, heart, left kidney, and in the lymph nodes. Cultures were easily obtained from the two testes. The parasites in the two testes, the lymph nodes and the left kidney were very numerous; they were of the same size as in the patient but their forms were more irregular.

Taxonomy of Histoplasma duboisii

It is rather evident that the fungus isolated from the patient of Dubois, Janssens, and Brutsaert possesses a good many features of its own when comparison is made with *Histoplasma capsulatum,* the agent of classical histoplasmosis. First of all, very large forms have been encountered in man and in guinea pigs which have never been encountered in man or animal parasitised with *Histoplasma capsulatum:* to this morphological aspect of the new parasite we gave the name of *duboisii* form. In the guinea pigs, a parasitic form which seems identical with the parasitic form of *Histoplasma capsulatum* precedes the appearance of the *duboisii* form; we gave to this morphological aspect of *Histoplasma duboisii* the name of *capsulatum* form. There is every reason to believe that the *duboisii* form evolves from the *capsulatum* form. As a matter of fact *capsulatum* forms which are the first to appear in the testes of the guinea pigs inoculated with *Histoplasma duboisii* enlarge progressively, their capsule becomes thicker and thicker and finally they attain the typical aspect of the *duboisii* form. The cultures of *Histoplasma duboisii* possess also characteristics of their own, the main one being the perfectly round form of the chlamydospores; piriform chlamydospores never being produced as is the case with *Histoplasma capsulatum.*

I am not yet able to decide whether *Histoplasma duboisii* is less pathogenic than *Histoplasma capsulatum.* Both parasites do not seem to possess a very large pathogenic power for animals. This aspect may be modified when we are able to obtain the yeast phase of *Histoplasma duboisii,* which has not yet been possible.

FIGS. 5, 6. 5. *Histoplasma duboisii.* Culture 20 days old showing numerous macroconidia and microconidia, × 285. 6. *Histoplasma duboisii.* Culture 20 days old showing four echinulate chlamydospores and numerous microconidia, × 880.

For the different reasons explained about, I thought it justifiable to create the new species *Histoplasma duboisii* Vanbreuseghem 1952. It seems from the citations made in the beginning of this paper that this species had been discovered by other workers but it had been generally considered to be *Histoplasma capsulatum*. Duncan was the sole author to suggest that it might be a new species. We will learn in the future if I was right or not and if there is sufficient reason to admit that there exists in Africa, besides the classical histoplasmosis of which *H. capsulatum* is the cause, another histoplasmosis, African histoplasmosis, produced by *Histoplasma duboisii*.

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FURTHER STUDIES ON THE FRUITING OF PHYSARUM POLYCEPHALUM

WILLIAM D. GRAY [1, 2]

(WITH 2 FIGURES)

In earlier papers (Gray, **1938**, **1939**) the writer demonstrated that plasmodia of the Myxomycete, *Physarum polycephalum* Schw., usually did not form fruiting bodies in the absence of visible light. From preliminary experiments conducted with radiations which were predominantly in narrow ranges of the visible spectrum it appeared that the shorter wave lengths of visible light were more effective in the initiation of sporangia formation than were the longer wave lengths. The work herein reported is an extension of the earlier work, employing light of known intensity from narrower regions of the visible spectrum.

EXPERIMENTAL PROCEDURE AND RESULTS

In these studies stock cultures were maintained by the moist chamber method described by Camp (**1936**), but the actual test cultures were starved cultures in beakers prepared as described in a previous report (Gray, **1939**). All experiments were conducted at 25° ± 1° C, and the initial pH value of the liquid in the beakers (McIlvaine's buffer, diluted 1 : 10) was 3.0.

As a source of light, a large mercury vapor lamp (with glass tube) was used, the radiations of various wave length being isolated by the insertion of a series of liquid filters between the lamp and a black paper lined chamber in which cultures were irradiated. The filter chamber series was built as follows: Four brass separators were made by bending square brass tubing (about ½ inch

[1] Department of Botany, The Ohio State University, Paper No. 552.

[2] The experimental work here reported was done during the writer's tenure as a National Research Council Fellow at the University of Wisconsin under the sponsorship of Prof. B. M. Duggar.

thick) into 19 × 19 inch squares, the open corners of which were brazed. Two ¼ inch holes were drilled through the tubing on one side and 3 inch lengths of ¼ inch (O.D.) brass tubing were soldered into each hole. The four brass separators were then laid alternating with five 20 × 20 inch squares of clear window glass, and wood frames were built to hold the glass panes, separated by brass squares, in position. The glass panes protruded about ½ inch beyond the brass separators all the way around, and the spaces between the protruding panes of glass were filled with a lanolin–resin glue, the preparation of which is described in a paper by Boeshore and Gray (**1936**). Thus, four watertight chambers (18 inches square and about ½ inch deep) resulted. Each of the chambers could be filled or drained independently of the others by means of the two round brass tubes which passed through the brass separator which formed the sides of that particular chamber.

Three wave bands of the mercury arc were employed: the blue line at 4360 Å, the green line at 5460 Å, and the two yellow lines at *ca.* 5780 Å. Liquids used in the filter chambers for the isolation of the blue and yellow lines were those recommended by Warburg (**1928**). The combinations of liquids used for isolation of the various wave bands were as follows:

ISOLATION OF THE BLUE LINE AT 4360 Å:

Chamber 1: 2% quinine hydrochloride
Chamber 2: 0.003% acid rhodamine
Chamber 3: Ammoniacal $CuSO_4$ prepared by adding normal NH_4OH to M/10 $CuSO_4$ until precipitation was complete
Chamber 4: 6% $CuSO_4$

ISOLATION OF THE GREEN LINE AT 5460 Å:

Chamber 1: 6% $CuSO_4$
Chamber 2: 0.22% $K_2Cr_2O_7$
Chamber 3: Neodymium ammonium nitrate solution
Chamber 4: Neodymium ammonium nitrate solution

The neodymium ammonium nitrate solution used in Chambers 3 and 4 in the isolation of the green line was not a solution of the

pure salt. The solution was prepared by heating 50 gms of a rare earth mixture (containing about 22 percent neodymium) in nitric acid on a steam bath until most of the liquid was evaporated; ammonium hydroxide was then added and most of the liquid was again evaporated. The process of heating first with nitric acid and then ammonium hydroxide was repeated several times until the soluble neodymium salt was formed. The soluble salt from 50 gms of rare earth mixture was dissolved in 325 ml of distilled water. When the absorption spectrum of this solution was examined, it was found that a 2 cm layer absorbed the yellow lines of the mercury arc but readily transmitted the green line. In addition to neodymium the rare earth mixture contained samurium, europium, and praseodymium; soluble salts of the latter probably aided in isolation of the green line, since praseodymium salts also transmit green light but absorb yellow light.

ISOLATION OF THE YELLOW LINES AT 5770 AND 5790 Å:

Chamber 1: 2% quinine hydrochloride
Chamber 2: 0.2% tartrazin plus 0.02% erythrosin
Chamber 3: 6% $CuSO_4$

Light intensities were measured by means of a thermopile–galvanometer system which had been calibrated against a standard lamp.

EFFECT OF WAVE LENGTH OF LIGHT UPON FRUITING. The effects of light of the above wave lengths upon percentage of fruiting and length of time necessary for fruiting (measured from the time at which the culture was placed under the light to the time that sporangia were mature) were ascertained by continuously irradiating beaker cultures of plasmodia with light passed through one of the liquid filter series. Results of these studies are presented in TABLE I.

From the results presented in TABLE I it is evident that sporangia formed in a greater number of cultures and the time necessary for the initiation and completion of the fruiting process was less when plasmodia were exposed to the shorter wave lengths of the visible spectrum. The possibility exists that, since the intensity of the radiation at 5460 Å was somewhat less than that at

TABLE I

THE RELATION OF FRUITING OF PHYSARUM POLYCEPHALUM TO WAVE LENGTH OF LIGHT

Wave length	Intensity (erg/cm²/sec)	Number of cultures	Number that fruited	Percentage fruiting	Average no. of hrs. necessary for fruiting
4360 Å	288	61	55	90.1	51.4
5460 Å	224	17	14	82.3	76.0
5770–5790 Å	392	38	9	23.7	120.0

4360 Å, the difference in time necessary for fruiting is not significant. However, the intensity of the radiation at 5460 Å was only about 22 percent less than at 4360 Å, whereas the length of the time period necessary for fruiting was about 48 percent greater in the cultures irradiated with the light of longer wave length. In an earlier paper (Gray, 1938; see Tables 1 and 4) it was shown that a 50 percent reduction in light intensity resulted in only a 7 percent increase in time necessary for fruiting. Thus, the differences reported for the cultures irradiated with blue light and green light cannot be due entirely to slight differences in light intensity. The intensity of the yellow light employed was 75 percent greater than that of the green light, yet cultures irradiated with yellow light required a 58 percent longer time period for fruiting than those irradiated with green light. From this it would appear that wave lengths of the yellow region of the visible spectrum are considerably less effective than those of the green region.

Since visible light has such a marked effect upon this organism, it is obvious that the various light rays must be absorbed to a greater or lesser extent. In order to gain a possible explanation of the dissimilar effects of the different wave lengths, extracts containing the plasmodial pigment were prepared, and percentage absorption of various wave lengths of light was ascertained with a Bausch and Lomb Spectrophotometer. No attempt was made to obtain the pigment in pure condition, the solution being prepared by macerating 5 gms of fresh plasmodium in 25 ml of cold acetone and filtering the extract; thus the solution contained all of the acetone-soluble materials of the plasmodium.

Percentage absorption of different wave lengths of visible light by a 5 mm layer of this extract is presented graphically in FIG. 1. From the absorption curve in this figure it is apparent that the greatest percentage absorption occurs in the blue end of the visible spectrum. In view of the fact that the plasmodial pigment has an absorption spectrum of this nature a possible simple explanation of the results presented in TABLE I might be that a longer period of time is necessary for initiation and completion of spo-

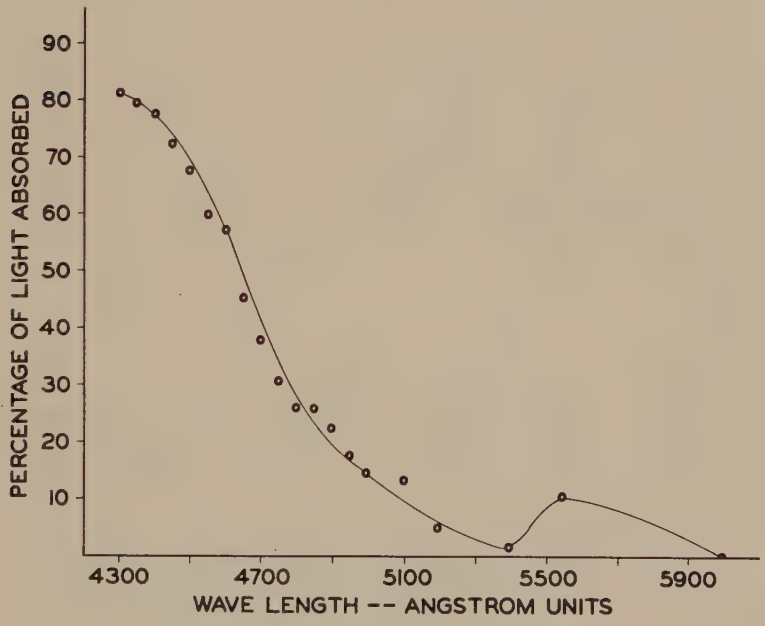

FIG. 1. The absorption of visible light of different wave lengths by an acetone solution of the pigment of *Physarum polycephalum*.

rangia-formation in the longer wave lengths because much smaller percentages of these wave lengths are absorbed. On the other hand, when percentages of yellow and green light absorbed are compared, there is further indication that qualitatively yellow light is less effective in this process than green light.

THE INTERRELATIONSHIP BETWEEN LIGHT ABSORPTION, pH AND FRUITING. In an earlier paper (Gray, **1939**) it was pointed out that in the pH range of 3.0–8.0, percentage of fruiting varies in-

versely with pH value, temperature and light intensity being constant. It was also reported that in the pH range 4.0–8.0, the length of time necessary for fruiting varies directly with pH value; i.e., the lower the pH value the sooner sporangia formed. Since the present work has shown that a relationship exists between fruiting and wave length of light, it was thought that perhaps the pH and light effects were in some way interrelated.

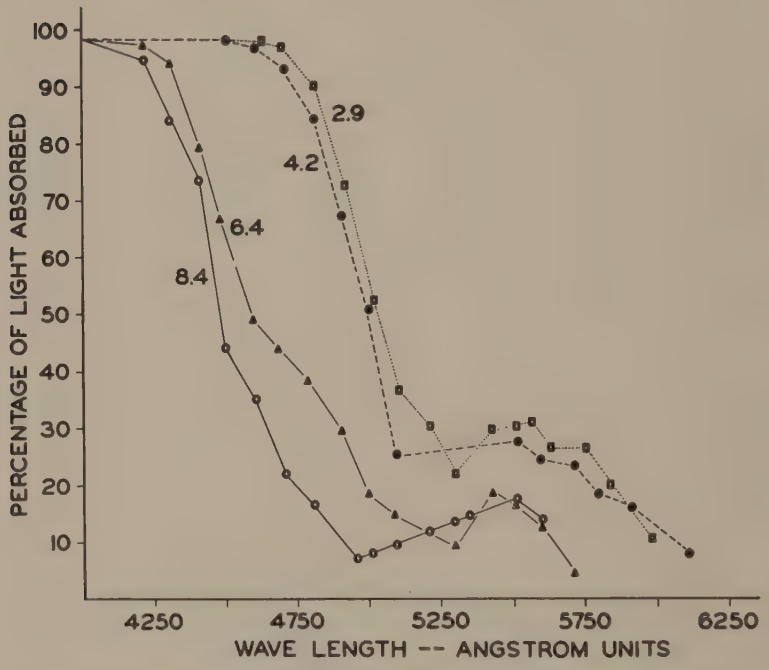

FIG. 2. The relation of pH to light absorption by the pigment of *Physarum polycephalum*.

Seifriz and Zetzmann (1935) have shown that the pigment of *Physarum polycephalum* is a natural pH indicator which changes color with pH changes in the surrounding medium. Since this is the case, it is reasonable to infer that changes in color which occur as a consequence of changes in acidity might also be accompanied by changes in light absorption. This point was investigated by preparing extracts by macerating 5 gms of fresh plasmodium in 25 ml of cold absolute methyl alcohol and filtering.

Five ml portions of such extracts were added to equal volumes of thallate buffer of various pH values, and the pH values of the resultant solutions were determined. The absorption curves which were obtained with 5 mm layers of four such solutions are presented in FIG. 2. From the curves presented in this figure it is evident that decreasing the acidity of the medium containing the pigment results in a reduction in light absorption by the pigment; this is especially noticeable in the violet and blue end of the visible spectrum. Such results provide a basis for the suggestion that the previously demonstrated pH effects may be due in part to the fact that increased acidity of the surrounding medium results in a change in the pigment in such manner that a greater quantity of light is absorbed by the organism. Such a course of events would inevitably lead to a more rapid initiation and completion of the fruiting process, since it has already been demonstrated that when other conditions are constant, time necessary for fruiting varies inversely with light intensity.

SUMMARY AND CONCLUSIONS

Plasmodial cultures of *Physarum polycephalum* Schw. were irradiated continuously with monochromatic light in the blue and green portions of the visible spectrum and a very narrow band in the yellow portion. It was found that the shorter the wave length of light the more rapidly the plasmodia formed fruiting bodies and the greater the percentage of cultures forming fruiting bodies.

When the absorption spectrum of a crude extract of plasmodium containing the pigment and other acetone soluble materials was determined, it was found that the greatest percentage of light absorption was in the blue end of the visible spectrum. Thus, the more rapid fruiting which was observed when plasmodia were irradiated with blue light of 4360 Å wave length was probably due to the fact that a much greater amount of light was absorbed, although there was some indication that longer wave lengths of light are less efficient in the initiation of the fruiting process.

Plasmodial extracts of different pH values had quite different absorption spectra: the greater the acidity of the extract, the greater the quantity of light absorbed; this was especially marked

in the range of 4000–5000 Å. It is suggested that the accelerating effect which increased acidity exerts on the fruiting process may be due in part to the increased capacity for light absorption by the plasmodium when placed in medium of low pH value.

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THE UTILIZATION OF NUCLEIC ACID DERIVATIVES BY NEUROSPORA

FREDERICK T. WOLF

(WITH 1 FIGURE)

The utilization of amino acids by microorganisms has received a great deal of attention during recent years. With respect to the nucleic acid derivatives, another group of naturally occurring nitrogenous substances, microbiological studies have centered largely upon the role of these compounds as accessory growth factors. Consequently, relatively little is known about the microbial disintegration of the nucleic acids, and the role of their degradation products as sources of nitrogen for growth.

Czapek (**5**) in 1902 performed some of the earliest experiments in which compounds related to the nucleic acid derivatives were tested as sources of nitrogen for fungi. Allantoin, alloxan, urea and uric acid were shown to be suitable nitrogen sources for *Aspergillus niger,* while barbituric acid and caffeine were less favorable for growth. Iwanoff (**10**) grew *A. niger* upon a medium containing thymus desoxyribonucleic acid as the sole source of nitrogen. After growth for 32 days, two-thirds of the nitrogen originally provided as nucleic acid was recovered in the mycelium of the fungus.

Kossowicz (**12**) cultivated *Aspergillus glaucus, A. niger, Botrytis bassiana, Cladosporium herbarum, Isaria farinosa, Penicillium glaucum, P. brevicaule, Phytophthora infestans, Mucor* sp., and *Fusarium* sp. upon media containing urea, uric acid, guanine, or guanidine·HCl as the nitrogen source, finding that all of these organisms grew well upon each of the compounds tested.

Brunel (**2**) found that uric acid and allantoin were satisfactory substrates for the growth of a large number of species of Basidiomycetes, including representatives of the Agaricaceae, Boletaceae and Polyporaceae. Employing enzymatic methods, he demon-

strated the conversion of uric acid to allantoin, allantoin to allantoic acid, and allantoic acid to glyoxylic acid and urea, by the enzymes uricase, allantoinase, and allantoicase respectively. In subsequent studies with *Sterigmatocystis nigra* (Brunel, **3, 4**) it was shown that allantoinase, allantoicase, and urease were constitutive enzymes in this organism, while uricase was not formed under any cultural condition. Recently Franke, Taha and Krieg (**9**) have made extensive studies of uric acid decomposition by a number of Phycomycetes (including species of *Rhizopus, Mucor* and *Cunninghamella*); Ascomycetes (including a number of species of *Aspergillus, Penicillium* and *Neurospora sitophila*); and Fungi Imperfecti (principally species of *Alternaria* and *Fusarium*), concluding that uricase is almost universally present as a constitutive enzyme among fungi.

Di Carlo, Schultz and McManus (**6**) have tested a large number of nucleic acid derivatives as nitrogen sources for the growth of the yeasts, *Saccharomyces cerevisiae* and *Torula utilis*. *Torula utilis* was able to grow upon media containing adenine, adenosine, adenylic acid, guanine, guanosine, guanylic acid, cytosine, uracil, xanthine, xanthosine, hypoxanthine, uric acid, or allantoin. It did not, however, utilize cytidine, cytidylic acid, uridine, uridylic acid, thymine, alloxan, ribonucleic acid or desoxyribonucleic acid. Thus the purine nucleosides and nucleotides were assimilated, while the pyrimidine nucleosides and nucleotides were not. *Saccharomyces cerevisiae* was able to utilize only adenine, guanine, cytosine and allantoin, of the substances tested, and thus was far less versatile than *Torula utilis*.

Kerr, Seraidarian and Brown (**11**) have studied the utilization of purines, purine nucleosides and nucleotides for ribonucleic acid synthesis in *Torulopsis utilis*, using precursors labeled with C^{14}. It was found that guanine could be formed from adenine, but this reaction was not reversible. While adenine and guanine were almost completely incorporated into ribonucleic acid, their nucleosides and nucleotides were utilized far less extensively for ribonucleic acid synthesis.

It is the purpose of the present work to study the utilization of nucleic acid derivatives and related compounds by the fungus *Neurospora sitophila*.

MATERIALS AND METHODS

The organism used in these experiments was a wild-type strain of *Neurospora sitophila*, "Beall" race, mating type A, originally obtained from Dr. B. O. Dodge, New York Botanical Garden (Dodge, 7). The basal medium used throughout the work was a modification of the minimal medium of Beadle and Tatum (1), in which ammonium tartrate and ammonium nitrate were omitted. Its composition was as follows:

KH_2PO_4	1.0 gm
$MgSO_4 \cdot 7H_2O$	0.5 gm
NaCl	0.1 gm
$CaCl_2$	0.1 gm
Sucrose	15.0 gm
Biotin	5 μ gm

Trace elements: Bo, 0.01 mg; Cu, 0.1 mg; Fe, 0.2 mg; Mn, 0.02 mg; Mo, 0.02 mg; Zn, 2.0 mg
Distilled water, to 1 liter

To this basal medium were added various nucleic acid derivatives in amounts calculated to provide 50 mg nitrogen per 100 ml. The pH was adjusted to 5.5, and the media were sterilized by autoclaving.

The media were inoculated with spores obtained from stock cultures grown on Beadle and Tatum's minimal medium. Cultures were grown in 125 ml Erlenmeyer flasks containing 25 ml of medium. All experiments were done in quadruplicate. After growth for 10 days at 30° C, the mycelia were removed by filtration, dried to constant weight in a desiccator over drierite, and weighed. In a few instances, it was necessary to repeat the experiments in order to obtain satisfactory agreement among the four replications. Controls were included containing no nitrogen, sodium nitrate, sodium nitrite, ammonium sulfate or asparagine as the nitrogen source, in order to facilitate comparison with the results obtained with nucleic acid derivatives.

The pH of all culture filtrates was determined at the end of the growth period. In all cases, except for ammonium sulphate and guanidine hydrochloride, the pH exhibited a trend toward greater alkalinity during growth, with final values ranging from 5.5 to 7.5.

The sources of the experimental chemicals were as follows: Orotic acid, thiouracil, thymine, hypoxanthine, theophylline, benzimidazole, adenosine, cytidine sulfate, inosine, uridine, xanthosine, adenylic acid, cytidylic acid, guanylic acid, and uridylic acid were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. Uracil, adenine sulfate, guanine hydrochloride, xanthine, allantoin, guanosine, ribonucleic acid and desoxyribonucleic acid were obtained from General Biochemicals, Inc., Chagrin Falls, Ohio.

Alloxan; barbituric acid; 2-methyl, 5-ethoxymethyl, 6-amino pyrimidine; uric acid; caffeine and urea were obtained from Merck and Company, Inc., Rahway, N. J. Cytosine, 5-methyl cytosine and 2,6-diaminopurine were obtained from Dougherty Chemicals, Richmond Hill, N. Y. Guanidine hydrochloride was obtained from Eastman Kodak Company, Rochester, N. Y., and 8-azaguanine was obtained from the Southern Research Institute, Birmingham, Ala.

TABLE I

GROWTH OF NEUROSPORA SITOPHILA IN MEDIA CONTAINING VARIOUS NUCLEIC ACID DERIVATIVES AS SOLE SOURCES OF NITROGEN

Nitrogen source	Mat weight
CONTROLS	
No nitrogen	0 mg
Sodium nitrate	112 ± 8
Sodium nitrite	0
Ammonium sulphate	23 ± 1
Asparagine	136 ± 6
PYRIMIDINES	
Alloxan[1]	0
Barbituric acid	6 ± 2.5
Cytosine	4 ± 1.5
5-methyl cytosine	5 ± 1
Orotic acid	6 ± 1
2-methyl, 5-ethoxymethyl, 6-amino pyrimidine	11 ± 2
Thiouracil[2]	13 ± 2
Thymine	4 ± 1.5
Uracil	4 ± 1.5
PURINES AND RELATED COMPOUNDS	
Adenine sulphate	82 ± 13
Guanine hydrochloride[2]	85 ± 5
Hypoxanthine	137 ± 12
Xanthine[2]	122 ± 16
Uric acid[2]	132 ± 12
Theophylline	12 ± 2
Caffeine	9 ± 1
Allantoin	119 ± 7
Urea	147 ± 5
Guanidine hydrochloride	42 ± 8

TABLE I—*Continued*

Nitrogen source	Mat weight
PURINE INHIBITORS	
Benzimidazole	0
2,6-diaminopurine[2]	0
8-azaguanine[2]	15 ± 3.5
NUCLEOSIDES	
Adenosine	130 ± 1
Cytidine sulphate	48 ± 12
Guanosine	133 ± 6
Inosine	137 ± 4
Uridine	14 ± 3
Xanthosine	134 ± 12
NUCLEOTIDES	
Adenylic acid	116 ± 3
Cytidylic acid	86 ± 9
Guanylic acid	127 ± 10
Uridylic acid	18 ± 9
NUCLEIC ACIDS	
Desoxyribonucleic acid[3]	105 ± 6
Ribonucleic acid[2,4]	106 ± 13

[1] Decomposed on autoclaving.
[2] Insoluble in the concentration used.
[3] The quantity used was calculated on the basis of 16.7% nitrogen content.
[4] The quantity used was calculated on the basis of 16.1% nitrogen content.

RESULTS

The results of the various experiments are presented in TABLE I, in terms of the weights of mycelia produced in media containing various nucleic acid derivatives as sole sources of nitrogen, in quantities equivalent to 50 mg nitrogen per 100 ml. In order to give some indication as to the reliability and reproducibility of the data, the standard deviations have been calculated from the individual mat weights found in the four replications of each experiment.

Because of the possibility of breakdown of nucleosides and nucleotides upon autoclaving, an experiment was done in which adenosine and adenylic acid were sterilized by passage through a Mandler filter, and added to basal medium which had been autoclaved. Under these conditions, mat weights obtained in adenosine averaged 134 mg as against 130 mg obtained on autoclaving, and with adenylic acid the values were 120 mg and 116 mg respectively. It was assumed, therefore, that destruction of other nucleosides and nucleotides upon autoclaving was negligible.

Since the studies of Woolley (20) had indicated that benzimidazole was a competitive inhibitor of purines in experiments with yeast, experiments were performed to test this substance as an inhibitor of purine utilization, with *Neurospora* as the test organism. In these experiments, hypoxanthine was used as the nitrogen source, at a concentration of 122 mg per 100 ml (corresponding to 50 mg percent of nitrogen), and the concentration of

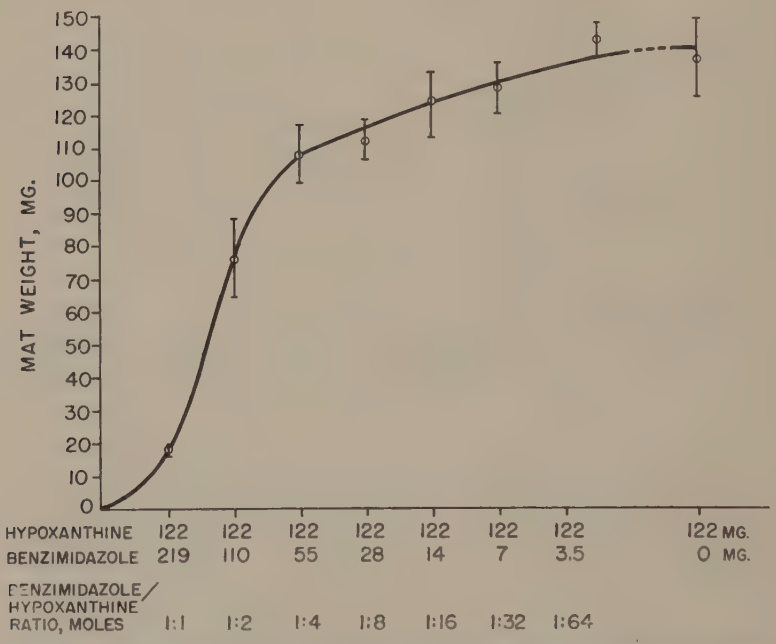

FIG. 1. The effect of varying amounts of benzimidazole upon the growth of *Neurospora sitophila*.

benzimidazole was varied. The results are presented in FIG. 1, in which each point represents the mean of four determinations, and the standard deviations are indicated by the lengths of the vertical lines passing through each point.

It may be noted that the growth of *N. sitophila* was almost completely inhibited by a benzimidazole/hypoxanthine ratio of 1 : 1, while the growth resulting from ratios of 1 : 16, 1 : 32 or 1 : 64 closely approximates that in the controls with hypoxanthine alone.

Fifty percent inhibition of growth was obtained at a benzimidazole/hypoxanthine ratio very close to 1 : 2.

DISCUSSION

In order to provide a standard of comparison for the nutritive values of the various nucleic acid derivatives for *Neurospora,* cultures were grown in media containing no nitrogen, or with sodium nitrate, sodium nitrite, ammonium sulphate, or asparagine as the nitrogen source. No growth occurred in the absence of nitrogen, or in sodium nitrite, the nitrite proving to be toxic in the concentration used. Growth was poor in ammonium sulphate because of the lowering of the pH of the medium to 3.8, a level unfavorable for growth. Sodium nitrate and asparagine proved to be excellent nitrogen sources for *N. sitophila.*

Pyrimidines proved to be very poor as nitrogen sources for *Neurospora.* Only a very small amount of growth occurred in the nucleic acid components cytosine, 5-methyl cytosine, thymine, or uracil. Barbituric acid, which has been recently shown to be a normal intermediate in pyrimidine metabolism, arising upon uracil oxidation by an unidentified soil bacterium (Wang and Lampen, **19**) and by *Nocardia corallina* (Lara, **13, 14**), supported only a trace of growth. Orotic acid has been shown to be able to substitute for uracil as a growth factor for a pyrimidineless mutant of *Neurospora crassa* and thus function as a uridine precursor (Mitchell and Houlahan, **16**), to be accumulated as a metabolic product in another pyrimidineless mutant of this species (Mitchell, Houlahan, and Nyc; **17**), and to be incorporated into the ribose nucleoside, orotidine (Michelson, Drell and Mitchell, **15**). It proved to be a poor nitrogen source for *N. sitophila* in the present experiments, as did alloxan, thiouracil, and the pyrimidine moiety of thiamine.

In contrast to the pyrimidines, purines were excellent nitrogen sources for *N. sitophila.* Good or excellent growth was obtained on adenine, guanine, hypoxanthine, xanthine, and uric acid. It is apparent from the data presented that a significant difference exists between adenine and guanine, the purine components of nucleic

acids, and the remaining purines tested, adenine and guanine being only about two thirds as effective nitrogen sources as hypoxathine, xanthine, or uric acid. The methylated purines, theophylline and and caffeine, are poor sources of nitrogen.

Allantoin and urea were excellent nitrogen sources for *N. sitophila*. The utilization by this fungus of adenine, guanine, hypoxanthine, xanthine, uric acid, allantoin and urea indicates the presence in this organism of the enzymes adenase, guanase, xanthine oxidase, uricase, allantoinase and urease. These findings suggest that the classical scheme of purine metabolism is operative in *N. sitophila*.

Three purine antagonists were studied; namely, benzimidazole, 2,6-diaminopurine and 8-azaguanine (guanazolo). No growth occurred in media containing benzimidazole or 2,6-diaminopurine as the nitrogen source, while slight growth was obtained upon 8-azaguanine. Drell (8) has recently reported that 2,6-diaminopurine, in relatively low concentrations, completely inhibits growth of a purineless mutant of *Neurospora crassa* in the presence of adenine or hypoxanthine, but not in the presence of adenosine or inosine. No previous work with benzimidazole or 8-azaguanine in connection with *Neurospora* has come to the writer's attention.

The data reported in FIG. 1 indicate that 50% inhibition of growth of *N. sitophila* was obtained at a benzamidazole concentration of 1.1 mg per ml. Woolley (20) reported half maximal inhibition of *Saccharomyces cerevisiae* at a benzimidazole concentration of 0.3 mg per ml. Further, from the data presented in FIG. 1, it may be noted that the benzimidazole/hypoxanthine molar ratio corresponding to 50% inhibition of growth of *N. sitophila* is approximately 1 : 2, indicating an inhibition index of 0.5.

It is further apparent that *N. sitophila* utilizes certain of the purine nucleosides and nucleotides for growth to a greater extent than it does the corresponding free purines. The amount of growth obtained upon adenosine, adenylic acid, guanosine or guanylic acid are significantly higher than those obtained upon adenine or guanine. Pierce and Loring (18) have concluded that the nucleosides play a more central role in nucleic acid metabolism in *N. crassa* than either the nucleotides or the free bases.

The differences in growth-promoting activity between adenine or guanine and their ribosides are not found to occur between hypoxanthine and inosine, nor between xanthine and xanthosine. It would appear to be of some significance to note that adenine and xanthine are constituents of the nucleic acids, while hypoxanthine and xanthine are not.

Among the pyrimidine nucleosides and nucleotides tested, little difference was found to occur among uracil, uridine or uridylic acid, all of which were poor nitrogen sources for *N. sitophila*. Striking differences were noted, however, among the cytosine, cytidine, cytidylic acid series, growth in cytidylic acid being 20-fold greater than in cytosine, and comparable to that obtained in adenine or guanine.

These experiments have been limited to ribosides and ribotides, and include none of the desoxyribose derivatives except for desoxyribonucleic acid. Desoxyribonucleic acid and ribonucelic acid are both utilized by *N. sitophila* as a nitrogen source, with equal effectiveness. As might be anticipated, the amount of growth resulting from these materials is intermediate between the high values obtained with certain purines and the low values obtained with pyrimidines.

The findings would indicate that *N. sitophila* is considerably more versatile, in its utilization of nucleic acid derivatives, than is either of the two yeasts studied by Di Carlo, Schultz and McManus (6).

ACKNOWLEDGMENTS

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SUMMARY

The utilization of 34 nucleic acid derivatives and related compounds has been studied in *Neurospora sitophila*. All of the pyrimidines tested (cytosine, 5-methyl cytosine, thymine, uracil, orotic acid, barbituric acid, alloxan, thiouracil, and the pyrimidine moiety of thiamine) were very poor nitrogen sources for growth of this fungus. The purines adenine, guanine, hypoxanthine, xanthine and uric acid, as well as allantoin and urea were excellent nitrogen sources for *N. sitophila*. Growth was poor upon the methylated purines, caffeine and theophylline.

N. sitophila utilizes certain of the purine and pyrimidine nucleosides and nucleotides (adenosine, adenylic acid, guanosine, guanylic acid, cytidine, cytidylic acid) for growth to a greater extent than is the case with the corresponding free bases. Comparable differences were not found between hypoxanthine and inosine, or between xanthine and xanthosine, all of which were excellent as sources of nitrogen for growth, nor among uracil, uridine and uridylic acid, which were poor nitrogen sources. Both ribonucleic acid and desoxyribonucleic acid are favorable nitrogen sources for growth of *N. sitophila*.

N. sitophila is unable to utilize the purine antimetabolites benzimidazole or 2,6-diaminopurine as a source of nitrogen, while 8-azaguanine is a poor nitrogen source.

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A FUSIDIUM DISEASE OF XYLARIA IN WISCONSIN [1]

M. P. Backus and E. A. Stowell

(with 10 figures)

An infectious disease affecting stromata of *Xylaria* has been encountered by the writers in the course of studies on various representatives of the Xylariaceae in southern Wisconsin. A single species of *Xylaria* has been found attacked in nature and in only one location, but the disease attained epidemic proportions in this situation. It first came to notice in the spring of 1951, although later examination of preserved specimens taken from the same site on previous occasions revealed that the pathogen was present here as early as 1945. The malady appeared to be highly destructive in 1951, and another local epidemic developed during wet weather in early June, 1952. It has been determined that the disease is incited by a fungus, *Fusidium parasiticum* Westendorp, which appears not to have been previously reported in North America.

THE HOST

The malady in question was encountered in a heavy stand of stromata of a species of *Xylaria* identified by Professor Julian Miller as *X. oxyacanthae* Tul. This is a species concerning which there has been considerable confusion and debate. Lloyd (4), Gilman and Tiffany (2), and others have considered it synonymous with *X. carpophila* (Pers.) Fr. The stand of stromata was located beneath a clump of hawthorns (*Crataegus mollis* ?) on the University of Wisconsin campus. In this location the *Xylaria* was first noted about 1944 and has reappeared each succeeding spring since that time. The stromata in this species are small,

[1] The work was supported by the Research Committee of the Graduate School of the University of Wisconsin from funds supplied by the Wisconsin Alumni Research Foundation.

often flattened, and frequently digitately branched above. Each stroma dug up by the writers has been found to be growing out of a buried hawthorn "seed" (nutlet), from which the mycelium apparently draws the major portion of its nourishment. In the vicinity of Madison, Wisconsin, the stromata of *X. oxyacanthae* usually make their appearance near the end of May. In 1951 there were many thousands of them present in an area of perhaps two square rods in the small hawthorn grove referred to above. Although slender and only about an inch high, the young stromata are conspicuous because of their light cream color; in large numbers, standing in close array, they present at this stage an arresting sight. After the production of conidia ceases, the stromata become dull grey, blending with the soil. The stromata of *X. oxyacanthae* make their appearance earlier in the growing season than do those of any of the larger Xylarias which are encountered in southern Wisconsin, and maturation of their ascocarps is more rapid. In about six weeks after the first appearance of the fungus above the soil, mature ascospores are present.

SYMPTOMS AND PROGRESS OF THE DISEASE

About the second week in June, 1951, small pure white spots were observed on a number of stromata which had been brought into the laboratory. These were full-grown stromata in which ascocarp development was under way and in which conidial production had declined. The surface of the stroma was becoming gray at this stage, and the white spots observed stood out prominently. Under a lens they appeared downy, resembling patches of downy mildew conidiophores; and microscopic examination revealed that they consisted, indeed, of dense masses of conidiophores and spores of an extraneous fungus. The number of stromata showing such patches soon increased until, particularly at one end of the stand, a very large proportion showed them. Furthermore, lesions of greater extent were now often encountered, some of them involving a large section of the affected stroma (FIG. 1). As the disease spread, it was noted that the *Xylaria* population declined, and close examination revealed many collapsed and withered stromata, shrunken down close to the soil

surface. By early July scarcely a hundred stromata could be found on the site. These remaining few were, however, firm and erect; and they contained mature asci.

The disease was slightly less severe in 1952, but its general

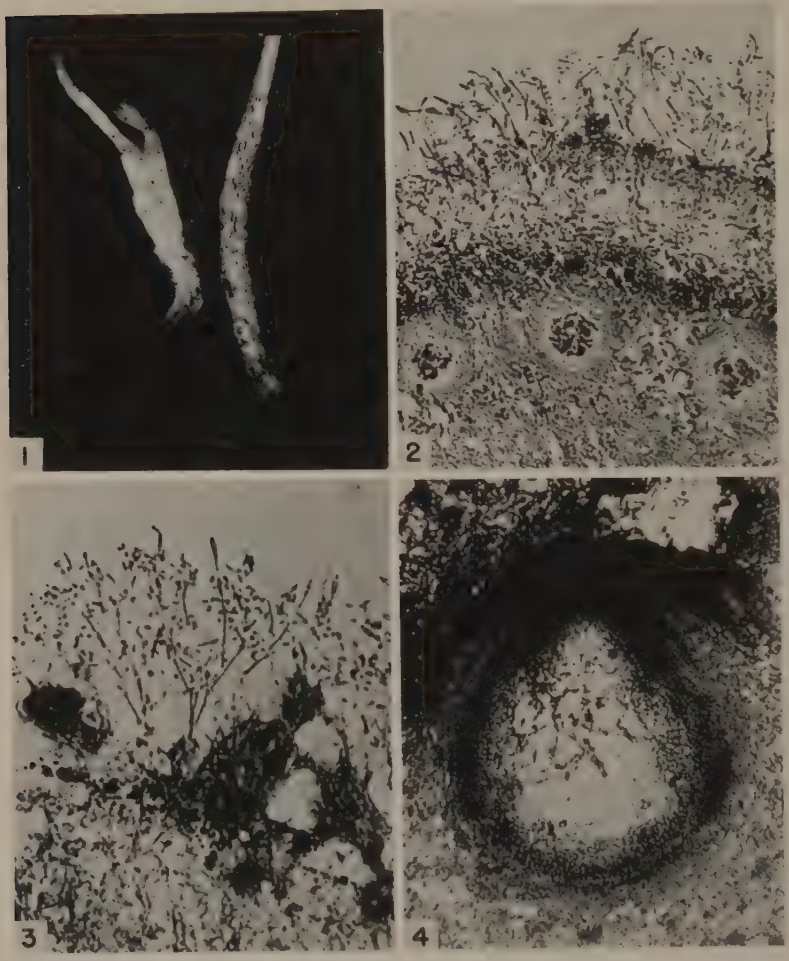


FIG. 1. Infected stromata of *Xylaria oxyacanthae* with white downy patches of *Fusidium* conidiophores, × 2. FIG. 2. Section through diseased stroma, showing conidiophores and invading mycelium of the parasite, × 165. FIG. 3. Branching conidiophores of *Fusidium* growing out from the conidial layer of the host. The dark areas represent masses of bacterial cells, × 195. FIG. 4. Aborted perithecium invaded by parasitic mycelium, × 170.

course was the same. In 1952 there was an early spring in Wisconsin, and the *X. oxyacanthae* stromata emerged nearly two weeks earlier than usual. Alerted to the possibility of a recurrence of the disease that had been prevalent the previous year, the writers kept close watch for the appearance of the characteristic stroma lesions; the first one was found on May 21. Thereafter the disease spread rapidly, and by the first week in June infected stromata were distributed throughout most of the stand. Again the *Xylaria* population rapidly declined, although the progress of the disease seemed to be temporarily arrested during a period of warmer weather which occurred about mid-June.

In *X. oxyacanthae* a certain proportion of the stromata are normally sterile, and these wither early, infected or not; but the impression was inescapable that in 1951 and 1952 the decimation of the *Xylaria* population in question was associated with a virulent infection. In the same location several years earlier thousands of stromata had been observed to come to maturity.

PATHOGONICITY STUDIES

The fungus forming white patches on the *X. oxyacanthae* stromata was successfully isolated by streaking conidia onto plates of 4 percent water agar and then picking out individual germinating spores with a sterile microscalpel under a high-powered stereoscopic microscope. Growth of this fungus on agar is very slow, but eventually some tube cultures were obtained which provided inoculum for pathogenicity tests.

Pure cultures of *X. oxyacanthae, X. polymorpha* (Pers.) Grev., *X. cornu-damae* (Schw.) Berk., and *X. acuta* Peck (?)[2] were available in the culture collection of the University of Wisconsin Department of Botany, these having been isolated several years previously by one of the writers. From them, cultures in cotton-plugged 500 ml Erlenmeyer flasks were now prepared. Previous experience in this laboratory having shown that autoclaved wheat grains provide a substrate favorable for stroma development, this medium was adopted for the flask cultures set up. The cultures

[2] The authors are indebted to Professor Julian Miller for this tentative identification.

were incubated on a laboratory shelf in diffuse light, since light had also been shown to be important for good development of stromata.

When the *Xylaria* stromata had reached nearly maximum size in the flasks, they were inoculated either by spraying them with a suspension of spores from tube cultures of the suspected pathogen, or by placing in contact with certain stromata small blocks of agar on which this organism was growing. The tests were repeated several times over a period of about nine months, two to four flask cultures of each of the four *Xylaria* species being included in each test.

In no case was infection of stromata of *X. cornu-damae* or *X. acuta* (?) accomplished. In a few instances very limited infection of stromata of *X. polymorpha* occurred in flasks inoculated by spraying. With *X. oxyacanthae,* infection was obtained by both methods of inoculation employed and was considerably more abundant than in the case of *X. polymorpha.* Typical white downy patches developed on the affected stromata, and the parasite was successfully reisolated.

On the whole, however, even in the case of the *X. oxyacanthae* cultures, infection was relatively sparse and the progress of the disease slow compared with that observed in nature. Various factors conceivably may have been limiting in the tests carried out. Only very light spore suspensions could be prepared from the scanty cultures which had been grown to provide inoculum; undoubtedly heavier conidial suspensions would have been desirable. Moisture conditions in the flasks very probably were not ideal: and the inoculations might have been more successful had the host been wounded, since some evidence has accumulated indicating that wounds may favor the entrance of the pathogen. However, the factor which very easily could have been the most important of all was the condition of the host. Stromata developed in cultures of the type used are far from normal. They are highly succulent; conidial production is sparse, and few if any ascocarps are initiated. Furthermore, the stromata do not, in appearance, closely resemble those produced in nature. For the most part they are much smaller and may not have the form characteristic of the species.

Curiously enough, those of *X. oxyacanthae* are typically larger than *X. oxyacanthae* stromata naturally produced; but they, too, are more or less abnormal in shape, and furthermore they usually develop in such a dense mass that their bases are in close contact or even fused.

HISTOLOGICAL OBSERVATIONS

Diseased stromata, collected in the field during both the 1951 and 1952 epidemics, were fixed in formol-acetic-alcohol and imbedded in paraffin. Microtome sections were stained with Heidenhain's iron-alum haematoxylin.

In the finished preparations, the mycelium of the parasite was observed growing throughout the stromata. The most profuse development appeared to be in the area of the conidiophore layer of the host, but hyphae were also commonly seen ramifying into the interior of the stroma among the massed hyphae of the host. They are readily distinguished from the *Xylaria* stromatic hyphae, primarily because they stain more heavily and because their nuclei are more prominent. In addition, the parasitic hyphae are generally of slightly greater diameter, and their orientation differs from that of the *Xylaria* filaments, most of which run parallel to the long axis of the stroma.

Interestingly enough, in the light of the field observations, most of the preparations examined showed surprisingly little damage to the host except in the area outside the ectostroma. Probably bearing on this situation is the fact that the specimens selected for fixation were mainly some which showed the conidiophores of the parasite in best condition; and it would now appear that the chief damage to the interior of the stroma must occur later. In any case there was little evidence of discoloration, shriveling, dissolution, or other degenerative changes of the ordinary stromatic hyphae in the region penetrated by the hyphae of the parasite. Furthermore, many instances were noted where the "Woronin's hyphae" of the young ascocarp initial appeared still to be in a healthy condition, even though numerous hyphae of the parasite were very close by. Occasionally normal and deteriorating initials were found immediately adjacent to one another. Thus, in FIG.

2 the dense uniform staining of the cells in the coils within the two initials at the left clearly indicates degeneration, while in the initial at right normal-appearing nuclei and cytoplasm could be observed in the more faintly staining fertile hyphae. Since in any healthy stroma a certain portion of the ascocarp initials regularly abort, it is difficult in the case of the parasitized stroma to be sure that any given degeneration represents damage caused by the fungal invader. Still, counts made in diseased specimens and in healthy specimens of approximately the same age do indicate that the number of aborting initials is considerably higher in the former. Furthermore, at a somewhat later stage, hyphae of the parasite have been identified actually growing through the interior of deteriorating young perithecia (FIG. 4) which had reached a point in development beyond that at which ordinary abortion usually occurs.

Various observations suggest that the ectostroma of the host is a barrier to the inward movement of the mycelium of the parasite. This stromatic layer, which is located a short distance below the *Xylaria* conidiophores, is composed of dark, closely-interwoven thick-walled hyphae. It is, however, a zone of rather uneven thickness and varying compactness. It may be that the parasite penetrates it only at its weakest points. Some small lesions have been noted where the mycelium appeared to be entirely exterior to this barrier; and it is quite possible that in some instances the pathogen never does succeed in breaking through into the interior of the host. Under natural conditions slugs, insects, etc. may cause breaks in the ectostroma, and such wounds undoubtedly assist the pathogen in getting into the interior of the stroma in some cases. Deep-seated infections have been noted in connection with some of these openings. The parasite can also gain easy entrance into the interior in another way, for infection may occur in the young upper portion of the stroma where the ectostroma is not yet differentiated.

Bacterial pockets (FIG. 3) have quite often been found associated with the fungal lesions. These pockets are sometimes of considerable size and may extend some distance into the entostroma. Conidiophores and stromatic hyphae of the *Xylaria* are

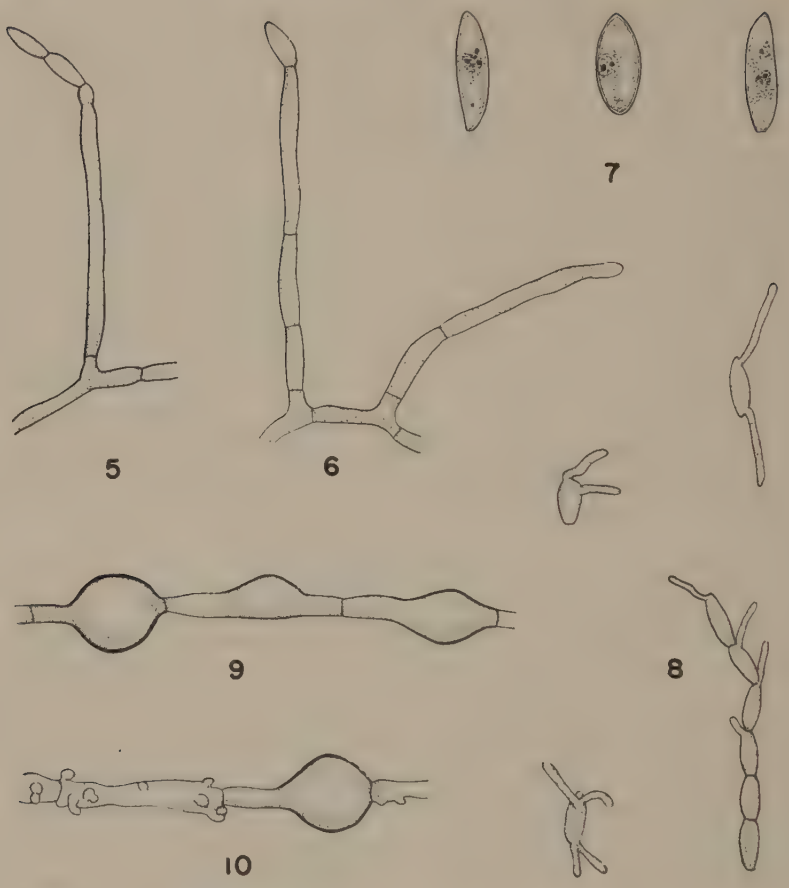
obviously dissolved by bacterial action in these areas, and the prepared slides reveal a solid mass of rod-type bacterial cells filling the lysigenous cavities. Since bacterial pockets have rarely been encountered except in association with the fungal lesions, it seems that the fungal parasite must somehow open the way for the bacterial invader. Wounds of the type which appear to facilitate entrance of the fungus into the stroma interior may also assist the bacteria in establishing themselves deep in the host tissue.

Fusidium conidiophores are produced in great abundance at the surface of the infected stroma, extending well beyond the shorter conidiophores of the host (FIGS. 2, 3). In microtome sections the conidiophores of the parasite were observed to be simple or sparingly branched, relatively coarse structures bearing unicellular fusoid conidia at their tips. The conidia (FIG. 7) and the cells of the conidiophores, like the mycelial cells, are uninucleate.

CULTURAL STUDIES

The fungal parasite was found to grow readily but very slowly on corn meal agar and on a *Xylaria* decoction agar. The latter medium was prepared using the extract of 25 gms of *X. oxyacanthae* stromata, 10 gms of dextrose, and 20 gms of agar per liter. On the corn meal agar, colonies showed an appressed, spreading growth reaching a diameter of slightly less than 2 cm in four weeks with incubation at 20° C. Conidiophores formed were mainly unbranched (FIGS. 5, 6) and were generally much shorter than those developed on the host, averaging only about 80 μ in length. They grew erect and bore long chains of hyaline, fusoid conidia. On the *Xylaria* decoction agar the growth was raised and cottony, but colonies 3–4 weeks old had a diameter of only a few millimeters. Here, too, however, spores were produced in moderate abundance. Conidia formed in culture were more or less spindle-shaped, averaging 11 × 3.6 μ. They corresponded closely in size and shape with conidia produced in nature, but much longer chains of spores were observed in the cultures than were ever found on diseased *Xylaria* specimens.

This fungus developed very poorly on a number of other common laboratory media on which an attempt was made to cultivate



FIGS. 5, 6. *Fusidium* conidiophores bearing conidia, from corn meal agar cultures, × 550. FIG. 7. Conidia, stained to show cytological detail, × 1100. FIG. 8. Germinating conidia, × 550. FIG. 9. Developing chlamydospores, from corn meal agar cultures, × 550. FIG. 10. Hypha with chlamydospore and with out-pocketings characteristic of growth on mannite agar, × 550.

it. Potato-dextrose, mannite, corn steep, honey-peptone, and Czapek's agars all proved to be unsatisfactory substrates. No production of conidia was obtained on any of these, and mycelial growth, when, indeed, it could be established at all, was extremely meager and abnormal. On the mannite agar [3] hyphal cells regu-

[3] Per liter: beef extract 3.0 gms; peptone 5.0 gms; mannitol 10.0 gms; agar 15.0 gms.

larly produced dwarf branches in the form of curious outpocketings (FIG. 10). These peculiar outgrowths have also been seen in cultures on corn meal agar and on *Xylaria* decoction agar, but much less frequently.

On most media used, chlamydospores were produced in the vegetative hyphae (FIG. 9); and these were especially abundant on the mannite agar. Similar spores have in rare instances been encountered in the hyphal mass from which the conidiophores arise on the *Xylaria* stromata. It is possible that they may play a part in carrying the fungus over winter.

Conidia produced in culture and conidia removed from diseased *Xylaria* stromata germinate readily on water agar and in hanging drops of distilled water. As a rule, one or two (rarely 3 or 4) germ tubes are produced. Spores from cultures may even germinate while still in chains (FIG. 8).

TAXONOMY

As noted above, the *Xylaria* pathogen in question appears not to have been previously reported in this country; but perusal of the foreign literature has revealed that an imperfect fungus closely resembling the Wisconsin organism is known in Europe.

Westendorp (7) in 1851 described *Fusidium parasiticum* from a collection or collections made by him on *Xylaria cornuta* in Belgium. He gave a brief but good description of the parasite and its appearance on the host; and he further recorded the observation that *Xylaria* stromata infected with this parasite almost never succeeded in coming to maturity. Spores of the *Fusidium* were described as about 1/50 mm long by 1/200 mm wide ($= 20 \times 5\,\mu$). Some forty years later Lambotte (3) reported another collection of this fungus on *X. cornuta* in Belgium. Additional collections from Belgium were recorded by De Wildeman (1) in 1898 with no description or comments included.

A similar fungus on *X. cornuta*, collected near Noidan, France (Côte d'Or) by F. Fautrey in 1890, is included as No. *5795* in Roumeguère's "Fungi Gallici Exsiccati". In the published account of fungi comprising Centurie LVIII (6), this organism is listed as *Fusidium parasiticum* West. forma *microconidia*, with the

notation "(10,12 × 3,3½ au lieu de 20 × 5)". It is assumed that these figures represent the dimensions, in microns, of the conidia of this parasite as contrasted with the measurements given by Westendorp in his original description of the species. Oudemans (**5**) lists this parasite on *X. cornuta* as *Fusidium parasiticum* West. forma *microconidia* Fautr., ascribing the form name to the man who collected the fungus in 1880 and who apparently prepared the notes on it for the above-cited French article.

Since Westendorp's observations were made at an early date, it seems possible that the measurements which he gave for the conidia of his *Xylaria* parasite could have been erroneous, and that Fautrey's fungus was really identical with the one described from Belgium on the same host. The Wisconsin material seems to correspond closely with the general description provided by Westendorp, but the size of the conidia coincides almost exactly with the measurements given for the fungus collected by Fautrey.

Specimens of *Fusidium parasiticum* on *Xylaria oxyacanthae* have been deposited in the University of Wisconsin Herbarium, the Farlow Herbarium of Harvard University, and in the Mycological Collections of the U. S. Dept. of Agriculture Bureau of Plant Industry; also in the cryptogamic herbaria of the University of Toronto, University of Michigan, University of California, University of Wyoming, and Washington State College.

SUMMARY

Fusidium parasiticum Westendorp, apparently previously unreported in the United States, has been found inciting a destructive disease of *Xylaria oxyacanthae* stromata at Madison, Wisconsin.

The fungus appears in late spring in the form of white, downy mildew-like lesions on the *Xylaria* stromata. As the disease progresses, the infected stromata become shrunken and disintegrate without maturing ascospores.

The fungus has been isolated and studied in pure culture. Of the various media on which cultivation of the organism was attempted, corn meal agar and *Xylaria* decoction agar supported the best mycelial growth, and these were the only substrates upon which sporulation was obtained *in vitro*.

Flask cultures of four Wisconsin species of *Xylaria* were inoculated with spore suspensions and mycelium from agar culture of *Fusidium parasiticum*. Moderate infection of the *X. oxyacanthae* stromata was obtained, and the parasite was reisolated from the lesions on the host.

In microtome sections through diseased *Xylaria* specimens the most profuse development of the *Fusidium* mycelium was observed to be in the area outside the ectostroma, but hyphae may penetrate to the very center of the host.

Bacterial pockets are commonly found associated with the *Fusidium* lesions. The bacteria involved are interpreted as secondary invaders.

Initial damage to the interior of the stroma is mild, even when the mycelium has penetrated deeply. There is, however, a marked tendency toward ascocarp abortion in later stages of infection.

The parasite produces septate, sparingly-branched conidiophores which bear long chains of hyaline, fusoid conidia. Haematoxylin-stained preparations revealed conidia, conidiophore cells, and cells of the mycelium to be uninucleate.

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THE DIAMETER OF THE MOLD COLONY AS A RELIABLE MEASURE OF GROWTH [1, 2]

F. P. Brancato [3] and N. S. Golding

(with 6 figures)

In an investigation in which the effect of certain controlled factors on the growth of an organism is to be considered, it is essential that the range of sensitiveness of the phenomenon chosen as an indicator of this influence be known. The diameter of the fungus colony has often been used as a measure of growth. In contrast, only a comparatively few investigators have attempted to evaluate this method of measuring fungus growth (Brown, 1922, 1923; Crozier, 1926; Worley, 1939). The object of this study was to determine the individual influence of some important environmental factors on the diameter attained by colonies of several common molds within a definite period of time, viz. rate of growth.

MATERIALS AND METHODS

The organisms used in this study were *Aspergillus niger* van Tieghem, *14*, and *A. flavus* Link, d; *Penicillium notatum* Westling, NRRL *832*, *P. expansum* Link, *69*, and *P. roqueforti* Thom, *33*; and *Geotrichum candidum* Link ex Loubière, m.[4]

Malt agar (Difco) was used as the basal medium. The des-

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[2] This investigation was supported in part by funds provided for biological and medical research by the State of Washington, Initiative Measure 171.

[3] Data from thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Bacteriology, Department of Bacteriology and Public Health, The State College of Washington. Present address is the United States Public Health Service Hospital, Seattle, Washington.

[4] Strains stored in the culture collection of the Department of Dairy Science, The State College of Washington, Pullman, Washington, under the code figures or letters following their names.

sicated medium was mixed with the required amount of distilled water and heated until completely dissolved. Twenty-five ml aliquots of this hot fluid medium were measured into 125 ml Erlenmeyer flasks by means of an autonomic delivery pipette. These flasks were autoclaved at 15 pounds pressure for 15 minutes (119° C). They were then cooled slightly and poured into sterile petri plates. The pouring was always performed within an inoculation chamber to minimize contamination.

The inocula for the agar plates were provided in the following manner: One ml of sterile distilled water was withdrawn from a 100 ml quantity and added to a 10-day-old malt agar slant culture. After mixing, as much of the water as possible was withdrawn and returned to the water blank. An L-shaped platinum wire was dipped into the resulting spore suspension. The center of the plated medium was pierced carefully with the tip of this moistened wire.

Unless otherwise specified, *A. niger* was incubated at 35° C for 3 days, *A. flavus* at 32.2° C for 4 days, *G. candidum* at 29.4° C for 7 days, *P. notatum* at 26.7° C for 7 days, *P. roqueforti* at 23.9° C for 7 days, and *P. expansum* at 21.1° C for 7 days. Except in the experiments on relative humidity, saturated aqueous solutions of $(NH_4)_2SO_4$ maintained the relative humidity of the atmosphere in the incubation chamber at about 80 percent.

The average diameter in mm of the reverse of the colony was the measurement recorded. These averages were computed from two measurements at right angles to each other of quintuplicate colonies. Since the test organisms produced circular giant colonies, the difference between the two measurements rarely exceeded one millimeter. The very rarely occurring replicate colonies of questionable diameter were subjected to the "Q" test, a simple screening test suggested by Dean and Dixon (**1951**) for use with small numbers of observations.

EXPERIMENTAL RESULTS AND DISCUSSION

A. Influence of Temperature.

Data contributed by many workers over the past years indicate that there probably is an optimum temperature for maximum

growth of each fungus; on either side of this temperature are temperatures inhibiting growth completely (Ames, **1915**; Miller and Golding, **1949**). A temperature allowing growth may not be favorable for the initiation of growth (Brooks and Cooley, **1917**). Moreover, differences in humidity or in concentration of the substrate may alter the temperature optimum for maximum growth (Groom and Panisset, **1933**; Bonner, **1948**). It has been observed that the effect on an organism of one temperature of incubation

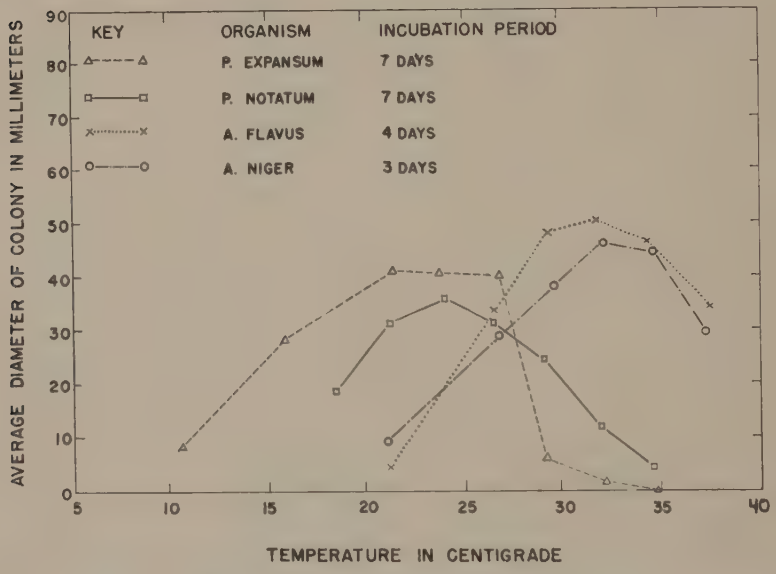

FIG. 1. Effect of variation in temperature on molds grown on malt agar.

may be discerned for a time after the organism is subcultured and incubated at another temperature (Fawcett, **1921**).

When four of the test organisms were grown over a wide range of temperatures, *A. niger, A. flavus,* and *P. notatum* yielded rather similar temperature-growth curves; *P. expansum,* however, exhibited some tolerance of temperatures lower than the optimum but a precipitous decrease in growth as the temperature rose above 26.7° C (FIG. 1). These growth curves are in accord with those obtained by Miller and Golding (**1949**) with the same organisms. All of these organisms have a favorable temperature range which

includes the optimum temperature and temperatures at which the diameter of the colony varies only a few mm from growth at the optimum temperature.

B. Influence of Concentration of the Medium.

In much of the work done on the influence of the concentration of the medium on fungus growth, dry weight data have been used as the measure of growth (von Mayenburg, **1901**; Weimer and Harter, **1921**; Wenck *et al.*, **1935**; Burkholder and McVeigh, **1940**, to mention a few). Where the diameter of the colony was used as the measure of growth, only the influence of small quantities of concentrating substances was studied (Golding, **1926**; Angell, **1929**; Galloway, **1935**).

TABLE I

EFFECT ON DIAMETER[1] OF MOLD COLONY (MM) OF ETHYLENE GLYCOL AND SODIUM CHLORIDE

Substance	Molal conc.	Organisms					
		A. niger	*A. flavus*	*G. candidum*	*P. notatum*	*P. roqueforti*	*P. expansum*
Molal concentrations of sodium chloride	0.00	38.3	52.9	80.6	32.6	63.6	38.0
	0.24	67.2	81.1	78.4	43.9	59.2	44.0
	0.47	57.2	72.7	56.1	44.5	47.3	40.2
	0.70	37.4	63.1	36.1	44.0	36.2	33.8
	0.94	28.5	54.8	18.0	41.8	25.8	28.8
	1.41	18.0	42.0	00.0	35.1	9.4	13.6
Molal concentrations of ethylene glycol	0.00	65.3[2]	52.7	80.2	31.0	53.7	35.1
	1.61	32.8	32.0	8.3	22.0	17.0	18.7
	3.32	4.5	6.2	0.0	1.8	5.3	1.0
	4.83	0.0	Germ.	0.0	0.0	Germ.	0.0
	6.44	0.0	0.0	0.0	0.0	0.0	0.0
	9.66	0.0	0.0	0.0	0.0	0.0	0.0

[1] Average of quintuplicate colonies.
[2] Incubated for 4 days.

The test organisms were grown on malt agar to which various quantities of concentrating substances were added. Glucose (Cerelose), sucrose (U and I), sodium chloride (Baker's Analytical C.P.), glycerol (Eastman Kodak), and ethylene glycol (Eastman Kodak) were used to concentrate the malt agar. FIGS. 2–4 inclusive and TABLE I illustrate the growth obtained when incuba-

tion was in the respective favorable temperature ranges of the organisms.

There was a stimulation of the rate of growth of all the molds by the lower concentrations of most of the substances. Ethylene glycol was the sole exception, exhibiting a marked toxicity which varied directly with its concentration. Although stimulation was most pronounced with the species of *Aspergillus*, the slope of the

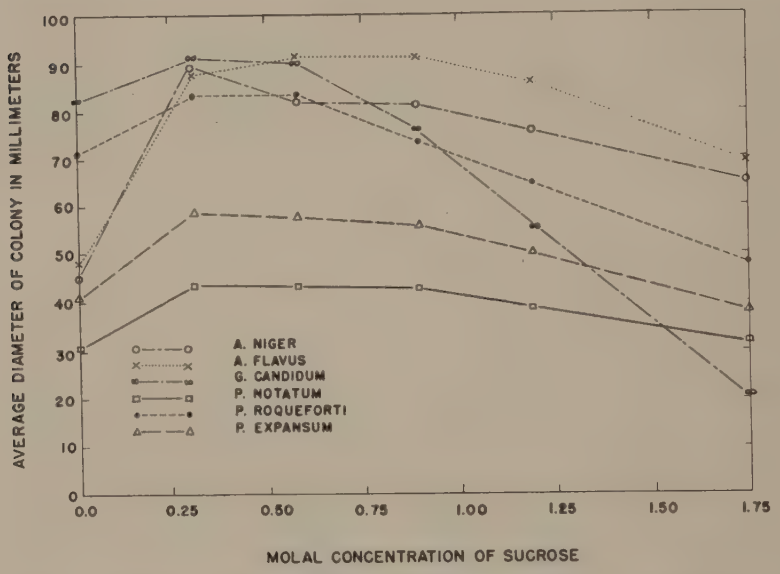

FIG. 2. Effect of variation in sucrose concentration on mold growth.

curve indicates that *P. notatum* was the most resistant to high osmotic concentrations. *G. candidum* showed the greatest inhibition by all the substances, and *P. roqueforti* was but slightly more resistant. The results of a study of probable causes of this stimulation will be reported at a later date.

C. Influence of Relative Humidity.

The importance of proper humidity conditions for the destructive growth of certain fungus plant pathogens such as the "powdery" and "sooty" mildews (Yarwood, **1935**; Fisher, **1939**), the rusts (Doran, **1922**; Stock, **1931**), the wood-destroying fungi

(Zeller, 1920) and many others has often been noted. Likewise development of moldiness on butter (Macy, 1929), on meat (Macara, 1943) and on textiles (Galloway, 1935) has been related to the occurrence of an atmospheric humidity suitable for mold growth. The effect of different relative humidities on the growth of various fungi has been studied chiefly on the basis of germination or non-germination of their spores or conidia. Rarely has the effect of different humidities on the diameter attained by the colony been recorded (Tomkins, 1929).

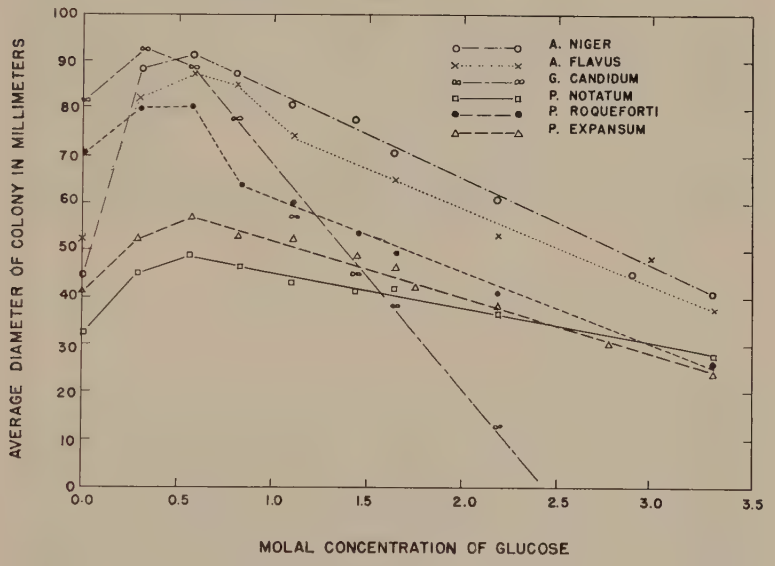

FIG. 3. Effect of variation in glucose concentration on mold growth.

P. roqueforti was used as the test organism in several experiments dealing with growth in atmospheres of various relative humidities. Desiccating jars were used as incubation chambers. In the first experiment there was no attempt to establish an equilibrium between the moisture in the substrate and the vapor pressure of the solution used in maintaining a constant relative humidity. TABLE II lists the humidity-maintaining substances along with the initial and the average relative humidities, as determined with a Serdex hygrometer, for the period of incubation. Also included in this table are the average diameters of colonies in dupli-

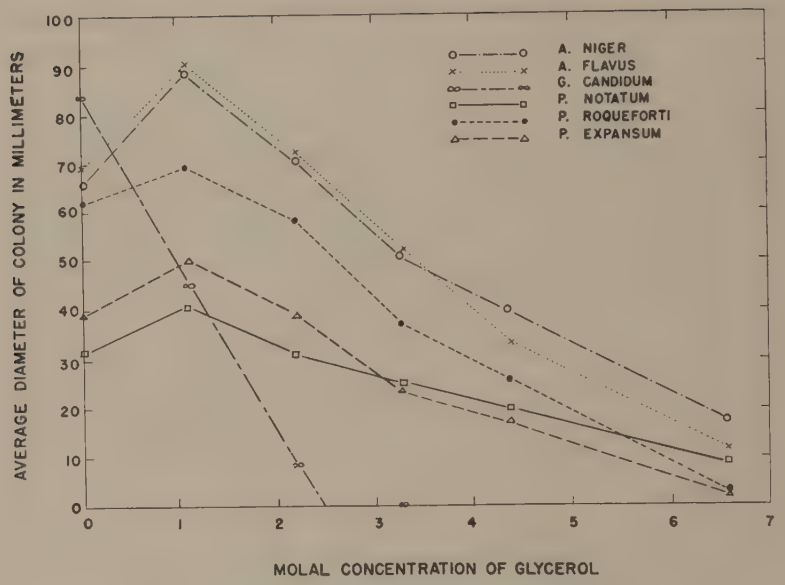

FIG. 4. Effect of variation in glycerol concentration on mold growth.

cate covered and uncovered plates. It is obvious that the relative humidity of the surrounding atmosphere had no influence on the diameter of colonies in the covered culture plates for the period of this experiment. FIG. 5 shows the morphological changes occurring at the higher relative humidity of closed plates. Similar results were obtained when uncovered plates were exposed to a relative humidity of 90 percent or higher. That the accumula-

TABLE II

DIAMETER OF P. ROQUEFORTI[1] ON MALT AGAR AT VARIOUS RELATIVE HUMIDITIES

Humidity-maintaining substance	Average relative humidity	Average diameter in mm[2]	
		Covered plate	Coverless plate
H_2O	98.0	70.0	72.5
Sat. $(NH_4)_2SO_4$ Soln.	82.3	74.0	75.8
44% H_2SO_4	48.7	73.3	42.3
54% H_2SO_4	36.1	74.5	26.3
Anhydrous $CaCl_2$	13.2	73.8	19.0

[1] Incubation for 7 days at average temperature of 25.5° C.
[2] Average of duplicate colonies.

tion of carbon dioxide in closed plates had no part in this morphological change was shown by absorption of this gas with NaOH solution.

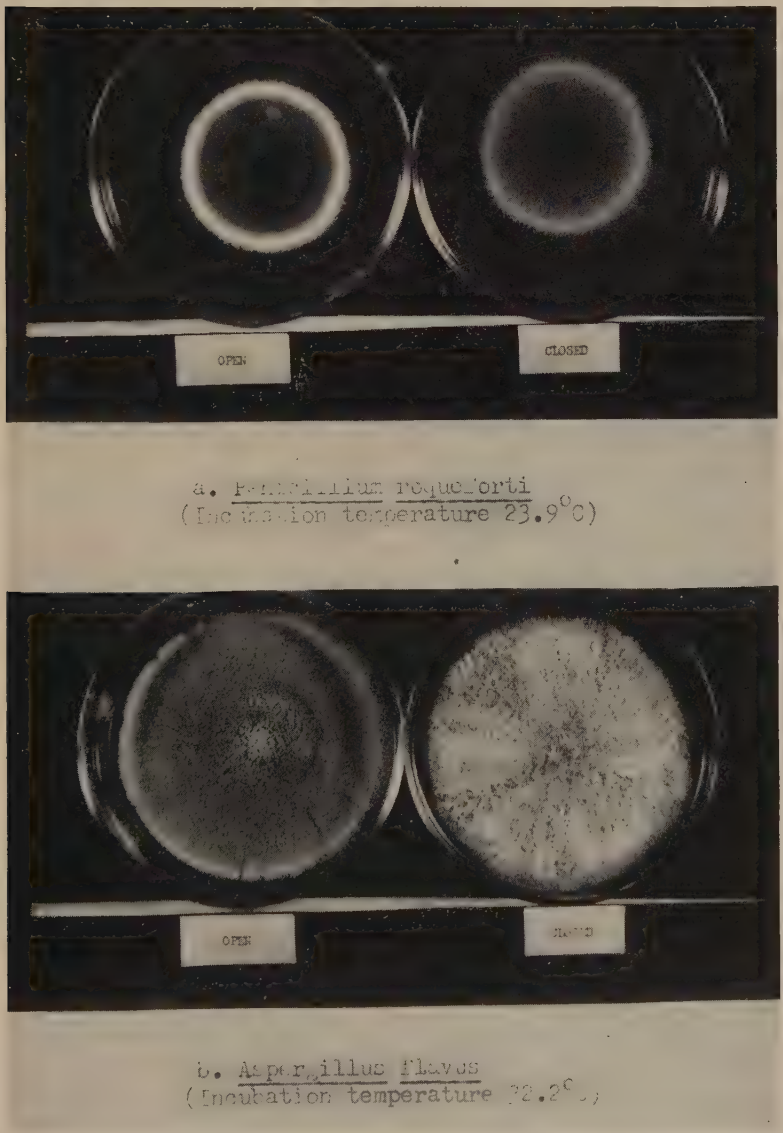

FIG. 5. Mold cultures grown on malt agar in covered and uncovered petri plates at a relative humidity slightly over 80 percent.

In the second experiment, uncovered plates of media were not inoculated until an equilibrium between their moisture content and the vapor pressures of various aqueous sulfuric acid solutions had been established. The range of relative humidities was from 60.7 percent to approximately 100 percent. It was observed that the relative humidity most favorable for growth was between 80 and 85 percent. This result is in accord with data obtained by other

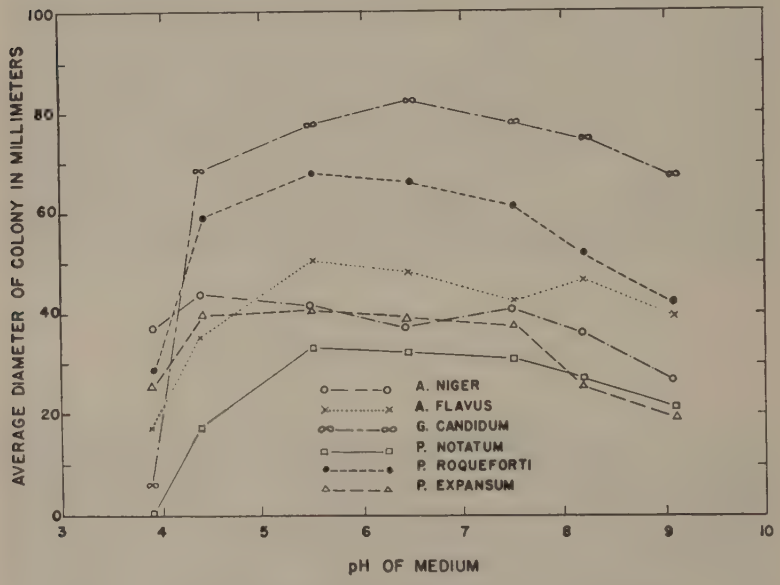

FIG. 6. Effect of initial pH of medium on mold growth.

workers with different species of Aspergilli and Penicillia (Lesage, **1895**; Tomkins, **1929**; Galloway, **1935**).

D. Influence of the Initial pH Value of the Substrate.

Investigators of the tolerance of fungi to varying hydrogen-ion concentrations agree that most of the fungi, and particularly those commonly called molds, have a broad region of pH insensitivity usually on the acid side of neutrality (Talice, **1930**).

In this experiment the reaction of sterilized malt agar (pH 5.5) was varied by the addition of sterile normal sodium hydroxide or normal hydrochloride acid. A Beckman glass electrode pH meter

was used to determine pH values. Quadruplicate plates of malt agar with initial pH levels of 3.9, 4.4, 5.5, 6.4, 7.5, 8.4, and 9.1 were inoculated with the six test organisms.

FIG. 6 presents the results as diameter in mm plotted against pH values. These curves with their broad plateaus of relative pH insensitivity show that the pH of the sterilized malt agar, 5.5, is optimum or very near optimum for all these organisms. *P. roqueforti* and *P. expansum* displayed the greatest and *G. candidum* the least sensitivity to alkaline substrates. *P. notatum* was the most sensitive and *A. niger* was the least sensitive to high hydrogen-ion concentrations.

The results of this experiment are in accord with the data reported by other investigators. Slight variations in the pH value of a medium between 5.5 and 7.5, occurring during its preparation, generally would not significantly influence the diameter attained by a colony.

E. Influence of Size of Spore Inoculum.

Brown (**1922**) studied the germination and growth of several common molds, including one species of both *Aspergillus* and *Penicillium,* under varied cultural conditions. He concluded that the density of the spore suspensions used as the source of inocula was most important when a weakly nutrient solution was used as the medium. On such a medium, growth diminished as the density of the spore suspension increased.

A study of the influence of the size of the spore inoculum upon the diameters of colonies of two of the test organisms was undertaken. For this experiment, *A. niger* was chosen as representative of molds which produce spores not readily wetted, and *G. candidum* was chosen because the preponderance of submerged growth in its colony suggested that its arthrospores were readily wetted. One ml aqueous spore suspensions were diluted with various amounts of sterile distilled water. Quintuplicate malt agar plates were centrally inoculated from each suspension in the usual manner, except that two of the plates were inoculated just before the agar solidified and then thoroughly shaken. Spore counts were made from the shaken duplicate plates after 24 to 36

hours of incubation at a temperature favorable for each organism.

TABLE III shows the two spore counts and the diameters of the three colonies for comparison. Under these experimental conditions, the diameter of the colony was independent of the size of the spore inoculum over a wide range.

TABLE III

COMPARISON OF SIZE OF SPORE INOCULUM TO DIAMETER OF COLONY ON MALT AGAR

Volume of spore suspension in ml.[1]	*G. candidum* (29.5° C)		*A. niger* (35° C)	
	Spore counts	Diameters in mm	Spore counts	Diameters in mm
25	65	58.5	19	46.0
	42	57.0	129	45.5
				45.5
50	62	58.0	83	45.0
	40	58.0	87	45.0
		58.0		46.0
75	34	58.0	15	45.0
	25	57.8	151	43.0
		59.8		43.0
100	44	58.0	49	43.5
	9	57.0	171	44.0
		58.8		44.5
150	46	57.5	22	43.0
	90	57.0	182	41.0
		58.0		42.5
Variation	9–90	57.0–59.8	15–182	41.0–46.0

[1] 1 ml aqueous suspension of spores diluted to various volumes with sterile distilled water.

F. Influence of Age of Inoculum Culture.

Methods for long-term preservation of culture or spore suspensions of fungi with a view to maintaining maximum viability have been described by many workers. It is apparent from these reports that the continued viability of many fungus spores is dependent on environmental conditions during dormancy or storage.

For the purpose of this study, it was desirable to know if any moderate variation in the age of the cultures used as sources of spore suspensions would influence the diameter attained by the

colony. Malt agar slant cultures of all the test organisms incubated at their respective favorable temperatures for 3, 11, 17, 22, and 30 days were used as source of inocula. Malt agar plates were inoculated in quadruplicate. The temperature and duration of incubation for each mold were the same used throughout this investigation. It was observed that there was no significant difference in the diameter attained by any of the molds that might be related to the age of the inoculum culture.

G. Influence of the Thickness of the Substrate.

Another step in the procedures used in this study, variations of which might conceivably have altered the diameter attained by

TABLE IV

RELATION OF THICKNESS OF SUBSTRATE TO THE DIAMETER AND DRY WEIGHT OF THE COLONY ON MALT AGAR

°C	Organisms	Depth of medium			
		4 mm		1 mm	
		Av. diam. in mm	Av. dry wt. in mg	Av. diam. in mm	Av. dry wt. in mg.
35.0	*A. niger*[1]	45.9	19.7	46.3	11.0
32.2	*A. flavus*[1]	52.4	33.7	52.7	3.6
26.7	*P. notatum*[2]	27.8	14.3	28.5	12.4

[1] Average of quadruplicate colonies.
[2] Average of duplicate colonies.

the colony, was the use of 25 ml aliquots of media as the standard substrate for culturing the molds. The influence of variations in thickness of the substrate on diameter of the colony was shown to be nil by conducing a simple experiment.

Twenty-five ml aliquots of malt agar were placed in ordinary petri plates (diameter 90 mm) and in large petri plates (diameter 140 mm). The resultant depths of the media at the center of these plates were approximately 4 mm and 1 mm, respectively. Two plates of each size were used in culturing each of three test organisms. After the diameters of the colonies had been recorded, the mycelial pads were removed by melting the agar and,

following a thorough washing with hot distilled water, their dry weights were determined.

TABLE IV displays the average of two runs with *A. niger* and *A. flavus* and of one run with *P. notatum*. It is obvious that although the thickness of the substrate did not affect the diameter, the dry weight of the colony was materially altered. This was strikingly apparent in the case of *A. flavus*. The smaller amounts of spores and of aerial mycelium formed by the molds on the thin substrate no doubt account for the differences in weight. These results exemplify the chief criticism of using the diameter of the colony as a measure of growth: that a true record of the amount of growth actually occurring is not obtained.

H. Influence of the Restricted Atmosphere of the Petri Plate.

One factor, namely the atmosphere over the colony, has not been discussed. There are several facts which necessitate a brief consideration of this factor. First, it must be recognized that gaseous exchange betwen the atmosphere surrounding the culture plate and the atmosphere within the culture plate is restricted; the partial pressures of oxygen and carbon dioxide within the plates have an inverse relationship, the former lower and the latter higher, to their partial pressures normally in air. Aqueous vapor pressure is also considerably higher within the plates. Second is the observation that petri plates used in this study differed in vol-

TABLE V

RELATION OF LIMITED ATMOSPHERE OF PETRI PLATE TO THE DIAMETER OF THE COLONY

Organisms[1]	Days of incubation	Diameter in mm[2]	
		Covered petri plate	Coverless petri plate
A. niger	3	47.0	47.0
A. flavus	4	55.5	54.5
G. candidum	7	83.0	83.0
P. notatum	7	34.0	36.5
P. roqueforti	7	63.0	56.0
P. expansum	7	34.0	36.5

[1] Incubated at temperatures favorable for particular organisms and at approximately 80 percent relative humidity.

[2] Average of duplicate colonies.

ume. The importance of this factor can be quickly dismissed with the words of Stock (1931) concerning uredospores of rusts and spores of other fungi that, "Das Sauerstoffbedurfnis für Keimung und Keimschlauchwachstum ist ausser-ordentlich gering." Moreover, during 7 days incubation, the longest any experiment lasted, neither the lack of oxygen nor the accumulation of carbon dioxide proved to be a limiting influence on the diameter attained by the colony (TABLE V).

GENERAL DISCUSSION

Some environmental factors which might influence the colony diameter attained by a mold have been tested and evaluated with the view of determining the effect of variation of these factors. Replicate colonies generally showed very close agreement. The rarity of questionable diameters and the good correlation add support to the use of the diameter of a colony for (1) measuring the rate of growth of fungi, (2) comparing the influence of variously altered media on their growth, and (3) investigating the effects of other environmental factors.

The diameter of a colony lends itself satisfactorily to the measurement of the growth rate because there is no acceleration of the growth rate with time. Within a short time after germination, many molds attain a characteristic radial growth rate at optimum temperature. This rate is maintained for a period of time varying with the organism and the medium. Preliminary experiments had shown that this was true of several of the test organisms. *P. notatum* attained and maintained a growth rate of 6 mm (range 5–7 mm) per day for 12 days, while *P. roqueforti* maintained a rate of 12 mm (range 11–14 mm) for 7 days, when the plates were overgrown. This steady radial growth rate is due to the fact that only the apical cells of the filaments multiply. Therefore the colony's growth rate may be considered a composite of the rates of three factors related to morphology and physiology: (1) the rate of division of the apical cells, (2) the rate of branching (i.e., increase in number of apical cells), and (3) the rate of enlargement of cells (Rahn, 1948). The fact that in some sectoring of fungus colonies the sector will disrupt the symmetrically radial

growth of the circular colony indicates that any one and probably all three of these factors may be gene-linked.

SUMMARY

The influence of temperature, concentration of the medium, relative humidity, reaction of the medium, size of spore inoculum, age of inoculum, thickness of the substrate, and atmosphere in the culture plate on the diameter attained by colonies of *Aspergillus niger, A. flavus, Penicillium notatum, P. expansum, P. roqueforti,* and *Geotrichum candidum* was studied. The results, in general, have been in accord with the data reported for other fungi and for different strains of the same species used in this work. Two of these factors, temperature and concentration of the medium, were observed to be important variables requiring careful control. The use of the diameter of the fungus colony as a measure of growth is considered sufficiently reliable for determining growth rates and for comparing the effect of environmental factors on a fungus cultured on the same medium. If an accurate knowledge of the the amount of growth is desirable, a cursory inspection of the giant colony will enable the researcher to judge whether or not this method of measuring growth introduced a gross error.

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FOUR YEARS OF MYCOLOGICAL WORK IN SOUTHERN SOUTH AMERICA

Rolf Singer [1]

With headquarters at the Instituto Miguel Lillo in Tucumán, the author has endeavored to collect fungi in the tropical, subtropical, temperate, and subantarctic zones of South America, to obtain data on the types of species already described from southern South America, especially southern Brazil, Paraguay, Bolivia, Chile, Uruguay and Argentina, to build up a South American reference herbarium for Basidiomycetes, and to try to coordinate the results obtained in a taxonomic-floristic study of the Agaricales of Argentina and South Brazil, with some observations on the general character of the basidiomycete flora of southern South America and its possible relations to other floras.

Collecting trips were organized, according to a preconceived plan, to the historical collecting places of Spegazzini around Bueno Aires, to the Sierras of Cordoba, to the subtropical and alpine regions of the provinces of Jujuy, Salta, Tucumán, and Catamarca, to the Chaco, to the region of the Selva Misionera (Misiones and Rio Grande do Sul), to the tropical coastal forest of Brazil from Paraná to Rio de Janeiro, and, on the other hand, to the mountains of Patagonia and to Tierra del Fuego in the *Nothofagus* zone of the South. In many of these regions, classical localities (Bruch, Spegazzini, Rick) were revisited; in others the author found himself to be the first visitor looking for Basidiomycetes, or fungi in general. These collections, together with material purchased or obtained by exchange, form part of the Cryptogamic Herbarium of the Lillo Institute at Tucumán, and represent the largest collection of South American Basidiomycetes, and the largest fungus herbarium in South America. Syntypes of most

[1] The content of this article coincides in essence with an address given by the author for the Sociedad Botánica Argentina at the Spegazzini Institute Eva Perón, the 9th of September 1952.

of the new species are preserved at the Herbarium of the University of Michigan, Ann Arbor, Mich. A summary of the collections in Argentina was given in a paper in joint authorship with A. Digilio,[2] and a review of the South Brazilian work may be found in a paper now in print.[3]

It seemed, however, useful and necessary to supplement the purely taxonomic treatment by a summary of the more outstanding results obtained, such as may throw a light on the character of the basidiomycetous flora of the territory now explored, on the problems which have arisen in connection with this exploration, and to add some personal experiences which might be useful for those who will, in the future, add more information on the subject.

The first impressions of a mycologist in and near the large cities (Montevideo, Buenos Aires, Eva Perón, etc.) of the Rio de la Plata region are somewhat conflicting until it is understood that the character of horticulture and agriculture here is essentially that of the northern hemisphere. Since the host frequently determines the fungous flora, one is likely to find, side by side with native elements, species unwittingly introduced by man, either in the vegetative form (mycorrhiza with seedlings of pines in the pine plantations of Uruguay, in poplar plantations along the Rio Paraná, in willow rows, on *Eucalyptus*, etc.), or else as spores attached to various materials, seeds, etc., or together with cattle, horses, and sheep introduced from Europe (dung-inhabiting fungi). Thus, we find commonly species of the genus *Suillus* in plantations, even *Amanita phalloides* and *A. muscaria*, have been introduced, together with such edible species as *Tricholoma flavovirens, Leccinum nigrescens, Lacturius deliciosus*, all apparently from Europe, while *Gymnopilus spectabilis* ssp. *pampeanus* (Speg.) Sing. was apparently introduced from Australia. While it is comparatively easy to recognize the adventive character of the parasitic and mycorrhizal species, it becomes exceedingly difficult to establish the origin of the humus and dung-inhabiting forms. We found *Camarophyllus pratensis* in its European form in the

[2] Singer, R. y Antonio P. L. Digilio. Pródromo de la Flora Agaricina Argentina, Lilloa **25**: 5–462. 1953.

[3] R. Singer. Type studies on Basidiomycetes VI. Lilloa **26** (in press).

parks of Greater Buenos Aires and the assumption was tempting to consider this as an example of an introduced humicolous species. Yet, later on, a form indistinguishable from the European *C. pratensis* showed up in the subtropical-montane forest near the borderline between Tucumán and Catamarca, where the entire vegetation was native, and no agricultural activities were going on nearby. The genus *Camarophyllus* was also found to be native in Patagonia. A similar example is that of *Macrolepiota procera.* Some Argentine mycologists had believed that the common large *Macrolepiota* of the "pampa" of Buenos Aires province is merely of European origin, or at least occupies an enormous area including Europe. This is not so. The Argentine species is different from the European one, although very close, and is certainly native, since it is also found in remote mountain meadows in the interior and on grassy places in the Chaco. It is *Macrolepiota bonaerensis* (Speg.) Sing. Even more difficult is the determination of the origin of the supposedly subcosmopolitan dung-inhabiting fungi, such as various representatives of the genera *Coprinus, Stropharia, Deconica, Bolbitius, Conocybe, Pholiotella,* etc. These species may have been cosmopolitan from the start, yet their present wide distribution can also be explained by the fact that cattle and horses are now found everywhere, especially in Argentina, and these animals are all descendants of introduced European stock. The occurrence of some of these species on the dung of native animals such as guanaco, birds, etc. cannot be taken as proof that they also existed here in precolombian times, since they may have invaded this habitat after introduction with cows and horses, donkeys and sheep. All these facts—and in addition misdeterminations of native species by the determining mycologists (Spegazzini, Herter, Rick)—may account for the predominance of European and American species in the floristic lists of South Brazil, Uruguay, and Argentina, and in the only book for mushroom amateurs published in South America (Talice & Talice) as well as in the exsiccati distributed by Herter.

The picture changes completely as soon as one enters the native woods and forests. Already at the southernmost extension of the Selva Misionera (reaching from South Brazil to Misiones and

along the Rio Paraná south to Punta Lara), the percentage of fungi common to South America and the extratropical regions of the northern hemisphere becomes very small. *Agrocybe aegerita* is apparently one of them, *Schizophyllum commune* another. *Mycena pura* appears to be a third example, and so does *Galerina clavata* and *Hydropus fuliginarius*. A certain number of pantropical or "American" species (among the former, *Oudemansiella canarii, Panus crinitus, P. siparius, Coriolus pinsitus, Pycnoporus sanguineus, Whitfordia elegans, Coriolopsis occidentalis,* and many others; among the latter *Heimiomyces tenuipes, Laccaria tetraspora, L. ohiensis, Xerula chrysopepla*) are easily recognized by those who have some knowledge of tropical fungi and of the eastern North American flora, especially that of Florida. Yet, the number of endemic species is undoubtedly large, although, with our growing knowledge of the tropical fungi of other continents, the neotropical and endemic element is bound to become gradually less conspicuous in lists of native South American species. Examples of truly native species of the La Plata region seem to be *Alnicola devia, Crepidotus tigrensis, Paxillus argentinus, Fricsula platensis,* but their precise area in South America cannot yet be circumscribed.

Traveling from the Rio de la Plata region northwest toward Bolivia, one passes the phytogeographic district of the Buenos Aires "pampa," which is a somewhat uniform stretch of almost treeless pastures characterized by rather abundant rainfall in spring and fall (April-June and August-September) and absence of snow, although freezing temperatures occur every year. Characteristic species inhabiting humus and dead herbaceous plants are *Amanita ameghinoi, Macrolepiota bonaerensis* and *M. kherandi, Volvariella cnemidophora, Marasmiellus caesioater, Marasmius pampicola, Clitocybe melliolens, Melanoleuca spegazzinii, Agaricus pampeanus, Bolbitius albiceps.* Common dung-inhabiting species are *Panaeolus subbalteatus, Anellaria sepulchralis, Agrocybe fimicola* (same as *Agrocybe coprophila* Sing. from Central Asia), which seem to be native, aside from many other species which may have been introduced but are now well established, such as *Stropharia semiglobata, Panaeolus phalaenarum,* etc.

Before the subtropical forest of the province of Tucumán is reached, one passes through the more or less xerophytic zone of the Chaco region (Chaco Santiagueño). This is a vegetation with scattered low trees, numerous cactaceous plants, and little or no herbaceous covering of the deep dry soil. There are entire seasons devoid of fungous growth because of absence of enough rainfall (except for occasional thunderstorms which provide enough moisture for a small number of saprophytic and parasitic Pyrenomycetes, Imperfecti, Gastromycetes, and half a dozen polypores, stereaceous fungi, and *Schizophyllum commune,* and a number of rusts and smuts, and hyphomycetous and phycomycetous soil fungi), but once in a while there is a period of prolonged (more than 3 days) rainfall and then an interesting fall flora of uniformly very small carpophores of agarics will appear, some of them with wide general distribution (*Conocybe lactea, Omphalina pyxidata, Crinipellis subtomentosa*), others, and this is the majorty, either endemic or at least restricted to the arid regions.

The forests of the so-called Selva Boliviano-Tucumana ascend in a characteristic zonation due to altitudinal climatic variations, the lowest belt being covered by a subxerophytic leaf-shedding forest with spiny undergrowth, forming some kind of transition between the Chaco formation and the subtropical rain-forest. Typical representatives of the basidiomycetous flora of this zone are *Tubaria omphaliopsis, Pluteus viscidulus, Leucoagaricus lilacinus, Lepista glabella,* various species of *Agaricus,* and *Marasmiellus, Fomitopsis sagraeana* (Mont.) Sing. (*Fomes sagraeana* Mont.), *Phellinus gilvus, Simblum sphaerocephalum. Heimiomyces tenuipes, Clitocybe scandens, Marasmius sanguirotalis, M. ruforotula, M. silvicola,* these and many others growing here are not limited to this zone, and even *Tubaria omphaliopsis* is often found in the drier zone of the montane *Schinus*-association. The subxerophytic zone reaches, in parts, deep into the subtropical zone along the ridges, whereas the true subtropical rain forest is most luxuriously developed in the narrow ravines ("quebradas") entering the Aconquija Mountains in a generally westerly direction. This forest is what may be called a "laurel" association (with *Phoebe porphyria* as the character tree). Here, we find a rich Basidiomycete-flora,

partly identical with that of the Selva Misionera to the far east, partly with species known to occur much farther north in the tropical forests of Orán, Bolivia and Perú, and the Upper Amazonas. Here again, the pantropical element is strongly represented (*Panus crinitus* and *P. siparius*, perhaps *Crepidotus citrinus* and *C. quitensis*, certainly *Diacanthodes philippinensis* and other polypores), while some species, according to our present knowledge, must be listed as endemics (*Phylloboletellus chloephorus, Marasmius bromeliacearum, Phaeomycena macrospora*, etc.). Species where the origin from farther north (up to Perú and Ecuador) can clearly be traced, are *Marasmius isabellinus, Crinipellis eggersii, Macrocystidia occidentalis*. "American" species are *Marasmius albogriseus, Crepidotus nephrodes* and *C. dorsalis*, again *Heimiomyces tenuipes*, and various others. Representative examples of species occurring here as well as in the Selva Misionera (without having, as far as we know, a much further extended area) are: *Gyrodon rompelii, Marasmiellus byssisedus, Marasmius hymeniicephalus, M. leoninus, M. magnificus, M. silvicola, Neopaxillus echinospermus, Calocybe cyanea, Armillariella puiggarii, Hydropus fuliginarius, Resupinatus subrhacodium, Collybia collybioides, Cantharellula coprophila, Rhodocybe himantiigena, Heimiomyces pruinatipes, Marasmius puttemansii, M. echinatus, Crepidotus acanthosyrinus, Hohenbuchelia paraguayensis, Lentinus puiggarii, Panus microspermus, Pleurotus rickii, Pluteus viscidulus, Favolaschia pygmaea*. Since the Selva Boliviano-Tucumana has been studied for basidiomycetous fungi for four years by the author and less intensely by others, it may be of some value to add a list of species which do occur in the Selva Misionera east to Porto Alegre, but have never been encountered in northwestern Argentina: *Clitocybe aberrantissima, Trogia cantharelloides, Callistosporium luteofuscum, Melanoleuca balansae, Chaetocalathus liliputianus, Crinipellis macrospaerigera, C. misionensis, Filoboletus gracilis, Hydropus riograndensis, Pluteus riograndensis, Macrocystidia carneipes, Marasmiellus microscopicus, M. fascicularis, M. niveus, M. subfumosus, M. subpumilus, M. tropicalis, M. vinosus, M. viridiluteus, Marasmius iguazuensis, M. ilicis-macrodonti, M. neosessilis, M. pallipes, M.*

trichorhizus, Xerula pilosa, Pholiota apiahyna, Hebeloma austro-americanum, Inocybe hyperythra, Galerina arenaria, G. minor, Crepidotus croceotinctus, C. palmarum, C. uber, Rhodocybe gilvoides, Leucopaxillus brasiliensis, L. gracillimus, Ripartitella brasiliensis, Lepiotella brunnea, Leucoagaricus rubrofibrillosus, Agrocybe retigera, Conocybe macrorhina, C. missionum, Xerocomus brasiliensis, Russula puiggarii, Russula riograndensis, Lactarius braunii, L. russula, Favolaschia rubra, F. puiggarii, F. sacchari-fera, Arrhenia pezizoides, A. cyphelloides, Brauniella albida, and, of course, all those species growing on *Sphagnum* (collections from Taimbesinho, Rio Grande do Sul) and *Araucaria* (such as *Tricholomopsis araucariae*). A list of species occurring in northwestern Argentina and north to Perú but not observed in southern Brazil would serve no purpose since in spite of the many collections from the latter region, there are still many species waiting to be discovered and added to the list published by us.[2]

Returning to the zonation of the Selva Boliviano-Tucumana, we ascend now from the true subtropical rain forest into the subtropical-montane belt, also called fog-forest, and dominated by myrtaceous trees. This zone is—at least mycologically—poorly and indistinctly separated from the lower zone, and there are only a few species worth mentioning as specific for this formation, usually occurring on *Eugenia* sp. or on *Chusquea,* which forms the underbrush: *Mycena micromphale, Rhodophyllus argentinus, Lentinus sayanus.*

The uppermost zone is markedly cooler than the others, and consists, as far as trees are concerned, of three associations, the coniferous woods (the lowest, in general, formed by *Podocarpus parlatorei*), the alder woods (the most extensive, formed by *Alnus jorullensis* var. *spachii*) and the "genoa" woods (generally the highest, bordering the subalpine meadows, formed by a rosaceous tree, *Polylepis australis*). This zone of montane woods with, (except for the numerous epiphytes and lack of a permanent snow cover in winter) almost boreal aspect, had hitherto never been visited by a mycologist or anybody collecting fungi. While the lower zones of the Selva are—with one exception, which we shall mention later—devoid of non-endotrophic mycorrhiza, the *Alnus*

woods show an abrupt predominance of Agaricales of the mycorrhizal type as generally common in regions where representatives of the Fagales and Salicales, *Pinus* and related conifers, *Tilia* and *Fraxinus* are common. While in the lower zones the average size of the fruiting bodies of the Agaricales is small, we find here some of the familiar "northern" type of fleshy fungi, one species of *Lactarius*, one *Gyrodon* (*G. montanus*, strangely a closest relative of the European *G. lividus*), three Russulas (quite different from the type of primitive tropical species represented in Venezuela and Brazil), at least two Cortinarii and one *Inocybe*. Naturally, we also find the genus almost regularly associated with *Alnus* formations and forming mycorrhiza with this tree, the genus *Alnicola* (with two species). It was *Alnicola diplocystis* which gave us first the necessary evidence of the presence of ectotrophic mycorrhiza in the Alnetum since the rootlets of *Alnus* from Catamarca were surrounded by the hyphae of this species (see Schweiz. Zeitschr. Pilzk. 28: 185. 1950 and Sydowia 4: 153. 1950). Only very few species of the lower belts are occasionally found in the *Alnus* woods, and even then they are rare, and evidently intruders (*Marasmius berteroi*, *Heimiomyces tenuipes*, the latter, as may be expected, being the most versatile of all species occurring under all possible conditions from extreme dryness in the subxerophytic forest to the extreme moisture of the South Brazilian jungle, and from tropical hot climate (Trinidad) to montane temperature zones, attacking any woody substratum living or dead from coniferous wood to monocots). On *Podocarpus*, we have found interesting isolated specimens of a northern genus, *Xeromphalina*, a species closely related to *X. campanella*, always occurring on conifers in the northern hemisphere, here replaced by *X. helbergeri*. On *Alnus*, we have a similar example in *Flammulina velutipes*, a winter-fruiting species of the northern hemisphere, never before observed in South America. Some of the non-mycorrhizal species occurring in the Alnetum also grow in pure stands of *Polylepis*, e.g., *Clitopilus argentinus*, *Clitocybe scandens*, and *Mycena pura*. *Polylepis* and *Podocarpus* do not form ectotrophic mycorrhiza, as shown by careful observations during three consecutive seasons. The same seems to be true for

Araucaria in Brazil and Patagonia, and for *Pilgerodendron, Fitzroya* and *Libocedrus* in Patagonia.

However, complete absence of mycorrhiza of the ectotrophic kind cannot be claimed for the subtropical forest. It is true that the genera of Agaricales usually forming the ectotrophic mycorrhiza in pine woods and in Fagales woods are absent; but there is one notable exception: *Gyrodon rompelii.* It is known that *Gyrodon lividus* and *montanus* form mycorrhiza with *Alnus,* and *G. merulioides* and perhaps *G. proximus* with *Fraxinus.* The tree-relation of *G. rompelii* remained a puzzle. Finally, it could be shown, by direct observation of tree roots and by a careful analysis of the tree flora around several populations of this species, that a new family of mycorrhiza-bearing angiosperms has to be added to the list: The Sapindaceae. The mycorrhiza host seems to be almost invariably *Allophylus edulis.*

Above the rain forest and the montane woods, we enter the subalpine meadows, and subsequently the alpine zone of the Pre-Andes and Andes. In certain regions such as the valley of Tafí (Tucumán) and Suncho (Catamarca), we encounter, aside from species more specific for this region (*Melanoleuca tucumanensis, Conocybe reticulatorugosa* and many other Conocybes, *Omphalina rustica*), various species already familiar from the "pampa" of the plains such as *Marasmius pampicola, Bolbitius albiceps, Macrolepiota bonaerensis, Agrocybe fimicola,* and *Anellaria semiovata.* In the alpine zone, from which no specimens had been known until now, a number of very interesting species were recovered, notably *Rhodocybe claudopus* in *Pycnophyllum-convexum* (Caryophyllaceae)-tufts, *Melanoleuca sparrei* and *Collybia sleumeri,* and finally, the species reaching higher than any other basidiomycete and at present holding the world altitude record: *Marasmius albogriseus,* collected by B. Sparre at 4250 meters in the Calchaquíes Range of the Preandine Sierras.[4] Naturally, in high-alpine regions with less precipitation, as in the alpine desert known here as Puna region, fungal growth becomes as rare and sporadic as phanerogamic vegetation, and only rarely an occasional

[4] Basidiomycete mycelium, apparently of *Hohenbuehelia pycnophylli* was collected by Sleumer in the province of Salta at 4500 m., February, 1953.

gastromycete is encountered. Only two Agaricales are known from such places, and as the time of fungus fructification here is not predictable, a methodical exploration of these regions is practically impossible. It is the same situation that makes us dependent on occasional collections by local inhabitants or travelers, here in the mountain deserts as well as in the great southern desert of western Patagonia. Nevertheless, even these random collections yield extraordinarily interesting material such as the type collection of *Galeropsis allosperma* Sing. (*Galera paradoxa* Speg.), the first and only species of *Galeropsis* without germ-pore, and therefore very significant in the interpretation of the phylogeny of Secotiaceae; for all Bolbitiaceae have spores with germ-pore, and the existence of a gastromycetoid form with spores without germ-pore, which in a group with thick complex spore walls is a relatively primitive character, can justly be used as an argument in favor of the derivation of the Bolbitiaceae from the genus *Galeropsis* rather than vice versa.

When judging lists of collections of Basidiomycetes from the various provinces of Argentina and Chile, from Paraguay and Brazil, northern Uruguay, and the eastern mountain slope of Bolivia, it should not be forgotten that some of the localities in seemingly very dry regions are actually marginal forests along smaller and larger rivers. It is these types of vegetation that form the "bridge" between the "Selva Misionera" and the "Selva Boliviano-Tucumana." Thus, a species common to both Selvas may also be found in marginal forests along rivers crossing the Chaco between them. A characteristic example is the interesting endemic genus of South America, *Neopaxillus*, with its only species *N. echinosperma*. *Salix humboldtina*, usually found in this type of vegetation, is often accompanied by its most conspicuous mycorrhizal partner *Hebeloma austroamericanum* and a lignicolous species, *Pholiota pseudofascicularis* (*Flammula alnicola* var. *salicicola*).

In order to coordinate the study of Rick's Brazilian types with collecting trips to the type localities and adjacent regions of the Selva Misionera where it is most luxuriant, two trips were organized, one to the Argentine northeast spur between Paraguay

and South Brazil, known as the territory of Misiones, the other, upon invitation by Dr. B. Rambo, S.J., to the Brazilian state Rio Grande do Sul. It is extremely difficult to find out about the collecting seasons in such regions, but acting on the best information available, we chose late spring and early summer in both cases. It seems, however, that basidiomycetous carpophores, even fleshy fungi, appear theoretically every month of the year, although, in spite of the frequent rainfalls, the summer crop of carpophores may be rather reduced because of possible short dry spells while such dry spells would not affect the necessary moisture minimum in winter or later fall; nevertheless, during winter, there is again the danger of cold spells (nights below freezing do occur) which stops the formation of carpophores for considerable time in some seasons; this is understandable since the majority of the Basidiomycetes, as we shall soon see, seem to be of tropical origin and have a rather high optimum and minimum temperature for mycelium growth as well as for carpophore production. More prolonged and careful observations would be necessary in order to determine whether or not there is a division into summer-fruiting and winter-fruiting Agaricales as could be observed in northern Florida, USA (see Farlowia 2: 97. 1945). Unfortunately, the Misiones trip was somewhat hampered by a dry spell and so was the Rio Grande trip; yet by visiting the moistest spots, swamps as well as the surroundings of waterfalls, a considerable amount of interesting material was gathered. Our impression is, however, that in spite of Spegazzini's short trips, our own two collecting expeditions, and the long period of years devoted to the collecting of fungi near Porto Alegre by Rick, the mycoflora of this best known section of Brazil is still apt to yield a large number of thus far unrecorded or misdetermined and too poorly described material. Rick did not believe in the value of dried material, so that many of his species are not represented in his herbarium, or else only by specimens hardly referable to the original concept of his species. The mycological flora of the Selva Misionera is incredibly rich. According to careful observations of Padre Rambo, the "campo" or grassland vegetation, characteristic for northern Uruguay, is gradually retreating, and the "selva" vegetation seems

to be gaining ground at the present time. This makes one think that the elements of the subtropical forest here consist mainly of species of tropical origin migrating from the north into Rio Grande do Sul, and carrying with them those species of fungi which are at the same time adapted to the hosts migrating south, and able to stand the climatic change (colder winters, occasional freezing temperatures although in most cases the microclimate in which the mycelium develops hardly ever reaches temperatures even near 0° C).

A few scattered collections by the author in the coastal tropical forest to the North of Rio Grande do Sul, especially in Paranaguá (State of Paraná, Brazil) and Angra dos Reis (State of Rio de Janeiro, Brazil), an analysis of the still existing material collected by various individuals and described by Rick, Spegazzini and Hennings from the states of São Paulo and Santa Catharina, Brazil, and finally the type material of some of Montagne's Brazilian species as well as those collected in the first half of the last century by Gardner and Spruce in Brazil—all these data taken together give a rather poor picture of the actual percentages of the mycoflora of both subtropical and tropical Brazil; in other words, it is impossible at present to check on Rambo's theory by an analysis of the existing mycological data. All we can do at present is indicate a number of species common to the tropical forests of Santa Catharina, lower Paraná and São Paulo, Rio de Janeiro, Minas Geraes, etc. on one hand and Rio Grande do Sul or Misiones on the other. No attempt is made to be complete. We merely give a few examples of such species on the basis of personal acquaintance with the material: *Collybia collybioides*, *Cantharellula coprophila*, *Marasmius magnificus*, *Marasmiellus eburneus* (Theissen) Sing. (*Marasmius eburneus* Theissen), *Leucoagaricus rubrofibrillosus*, *Russula puiggarii*. There is, on the other hand, no doubt, but that numerous species of the tropical Brazilian forest even south of the Amazonas, do not reach the South Brazilian subtropical forest, just as might be expected. Thus, there is no indication of *Marasmius ferrugineus* (unless it be identical with *M. spegazzinii*), *Marasmius albocapitatus*, and some Marasmielli, Collybiae, and Rhodophylli collected by me be-

tween Paraná and Rio de Janeiro, either among Rick's specimens at Porto Alegre or material collected by me in Rio Grande do Sul. Yet, there is naturally no proof that they do not occur at all. Recent papers of Dennis treating his agaric collections in Venezuela and Trinidad are a valuable addition to our knowledge of the fungous flora of northern South America, and one will encounter a large number of the species mentioned here, although some of Dennis' interpretations are not fully identical with those accepted in our papers. For example, what we call *Marasmius haematocephalus* is *Marasmius tageticolor* in Dennis, what we call *M. tageticolor* is an acystidiate species (the type) not mentioned in Dennis, and what Dennis calls *Marasmius haematocephalus* is a species unknown to me. What Dennis describes as *Marasmius aciculaeformis* is not that species, since the latter (type) has smaller spores while Dennis' species is probably *M. pallipes* Speg. His *Collybia cycadicola* is evidently *Lactocollybia angiospermarum,* and his *Micromphale rheicolor* is evidently our *Heimiomyces tenuipes.* His *Dictyoploca plectophylla* is either incorrectly determined (spores of type nonamyloid) or else there is a mistake about the amyloidity of the walls in the description, a circumstance which may also have led him to underestimate the value of this character in certain groups of Agaricales. *Marasmius leoninus* in the sense of Dennis is different from the type as well as from material of that species occurring in Brazil and analyzed by the author. *M. rhabarberinus* Berk., type, also differs from Dennis' concept of *M. leoninus* and from ours by having definitely free and more distant lamellae. These few remarks on an otherwise valuable and very instructive paper are necessary in order to show that in many cases even the comparison of the results of modern authors for comparative phytogeographical studies is rather risky, and may lead to wrong conclusions.

A particularly interesting phase of the mycological studies in Brazil was the study of fungi growing on *Araucaria,* and those found in the *Sphagnum* bogs of the higher altitudes in the Serra north of Rio Grande do Sul toward Santa Catharina. Dennis and I each found one representative of the genus *Tricholomopsis* in South America at approximately the same time, mine growing

on *Araucaria* in Brazil and being extremely close to the type species of the genus. Until then, the occurrence of this genus in thermophilous populations was only suggested by the existence of several Floridian representatives of the genus. The upper margin of the plateaus at about 1000 meters elevation showed very little Agaricales growth in otherwise seemingly very suitable conditions (mixed woods of *Araucaria, Podocarpus, Ilex* and other evergreens mixed in, with numerous tree ferns and mossy ground cover) in November. But the reason may have been a seasonal appearance of the Agaricales in this almost temperate climate with no previous experience as to the periodicity of fruiting in this particular region, which had never been visited previously by anyone collecting fungi. On the other hand, in the nearby *Sphagnum* bogs, a comparatively rich flora of sphagnophilous Galerinas, *Psilocybe uda, Collybia dryophila* var. *oedematopus* etc. was observed by us at the very same time, and it is likely that the flora of the *Araucaria* association in this altitude and latitude is comparatively poor. The character of the sphagnophilous fungous flora is decidedly boreal, especially as far as the acystidiate *Psilocybe uda* is concerned while, among the Galerinas, we found some evidently endemic species, a feature to be expected in a locality so isolated and peculiar in its flora components.

No mention is made of the pantropical or neotropical element and the cosmopolitan species. It would be easy to enumerate quite a few species found here as well as in northern South America, Cuba and Florida and in some cases also in tropical Africa and Asia. Particularly impressive by their numbers in every association studied and by their extremely common occurrence are *Schizophyllum commune, Pleurotus ostreatus* (cosmopolitan species) and *Filoboletus gracilis* (from Rio Grande do Sul north to Florida, and also outside America) and other species with similar distribution (*Collybia plectophylla, Dictyopanus pusillus, Coriolus occidentalis, Mycobonia flavida, Agrocybe aegerita, Panus rudis, P. crinitus and P. siparius, Clitopilus incrustatus, Rhodophyllus squamifolius, Oudemensiella canarii* and *steffenii, Polyporus tricholoma, Pseudofavolus cucullatus,* also many species of *Coriolus* and *Fomitopsis, Phellinus* and *Whitfordia*), and such American spe-

cies as the ubiquitous *Heimiomyces tenuipes, Crepidotus nephrodes* and *C. croceotinctus, Gymnopilus peliolepis* and *G. chrysopellus.*

Following the Andes and preandine sierras south, we find a wide xerophytic gap between the southernmost extension of the Selva Boliviano-Tucumana and the north tip of the *Nothofagus* woods of the South. This gap was studied mycologically by Spegazzini who received some scattered collections from the Cuyo region (including the important provinces of San Juán and Mendoza) and a rather large and complete collection made by Bruch in the province of Córdoba. Aside from a short trip to the latter province, we have not visited the treeless grasslands and deserts, the partly wooded mountain ravines and the parks and plantations of that region. Even a survey of the type studies on Spegazzini's material and our own experience does not warrant any attempt to enounce a theory as to the origin and character of this mycoflora. Its gastromycetous component is apparently rich and interesting; the agarics consist of some endemic as well as intruding elements from adjacent regions and of some species of wide distribution such as various polypores and *Bolbitius fragilis,* and the only bolete of the region, *Phaeogyroporus tropicus* ("hongo de Córdoba").

South of the nearly treeless gap, we find the Andes again covered by woods; in the northern part of West Patagonia intermixed with such more northern genera as *Chusquea* and with a rich myrtaceous flora, but basically, here as well as south to Tierra del Fuego, consisting of various species of *Nothofagus.* It is well known that the genus *Nothofagus* is one of the best examples of an area disjunction between Australia and Western South America. While the majority of the remaining phanerogamic vegetation does not show the same disjunction, it is important to note that the majority of the fungi are intimately linked with the most important host plants, the Nothofagi, and, consequently, show less kinship with the mycoflora of the central and northern part of the Andes and preandine Sierras than the phanerogamic flora. Before a full appreciation of this interesting aspect of the flora of Patagonia can be given, it would be useful to study what seems

to be the center of distribution of the basidiomycetous flora of this region, the woods of Valdivia in Chile. These woods have not been studied systematically thus far; only occasional collections from local collectors are available at Santiago, Chile, and in the Spegazzini herbarium. Earlier collections were sent to Montagne by Gay, and were published in Gay's famous *Historia Natural de Chile*. But this enumeration likewise contains only very few species so that it is necessary to depend on my own collections from east of the Chilean border and reaching to the borderline between Argentina and Chile. While a rich mycoflora is maintained in a coastal strip of land south of Concepción, Chile, to the south tip of the continent and then bending towards the east and reaching the Isla de los Estados (Staten Island), the mycoflora as well as the diversity of the *Nothofagus* species and associations formed by them becomes less striking while the sum of yearly rainfall decreases from west to east, and the margin of the woods is rarely very far from the Chilean frontier. East of the margin of the woods is the wide Patagonian desert, and only in the southern part of the Argentine sector of Tierra del Fuego do the woods reach the Atlantic coast. Rains, and with them Basidiomycetes, appear both in spring and—more abundantly—in fall, further south mainly in early fall.

Since *Nothofagus* belongs to the Fagales, there can be confidence as to which of the numerous large fleshy Basidiomycetes form ectotrophic mycorrhiza with these trees. Such mycorrhizal relations could indeed be demonstrated in the same way as they were first demonstrated in the Alneta of the Selva Boliviano-Tucumana. The latter, being the nearest wooded area with Fagales, was naturally suspected of having served as a bridge for the southward migration of species from the north tip of the area of *Alnus jorullensis* (Mexico) to the *Nothofagus* area. However, this idea turned out to be wrong: it is not borne out by the facts. The flora of the Alneta contains indeed very few species which also occur in the *Nothofagus* woods of the south according to detailed studies made by the author in numerous trips to the Alneta as well as in two extended expeditions to the *Nothofagus* area (Northwestern Patagonia: Neuquén and Rio Negro; Tierra del

Fuego: Rio Grande to Lago Fagnano). Leaving out of consideration such apparent cosmopolitan species as *Armillariella mellea* in the wider sense, *Clitopilus scepticoides, Schizophyllum commune, Mycena pura, Bjerkandera adusta, Laccaria echinosperma,* etc., the similarity between the Nothofageta and Alneta of South America boils down to the fact that they have altogether two species in common. One is *Crepidotus brunswickianus,* and the other is *Naucoria polytropa.* In the case of the latter, there even seem to be certain differences between the characters of the respective collections. More species may be added in years to come, but our present knowledge is certainly good enough to predict that the list of species common to the Nothofagetum and the Alnetum will never be large. There is likewise no resemblance between the Basidiomycetes observed on or under *Salix humboldtiana* further northeast and the same class of fungi observed in the *Nothofagus* area. *Laccaria tetraspora* (of wide distribution) and *Collybia platensis* seem to be the only species common to the two phytogeographic districts. On the other hand there is a definite relationship between the fungi of the *Nothofagus* area in South America and the fungi of New Zealand and Australia. Forms closely related to *Thaxterogaster* (South America) and *Descolea* (South America) have recently been observed by Heim in New Zealand; both Amanitae occurring in the *Nothofagus* area have their nearest relatives among Australian species (one seems to be identical with an Australian *Amanita*). We may add that the ascomycetous genus *Cyttaria* has actually the same distribution as *Nothofagus* itself. A better knowledge of the basidiomycetous flora of New Zealand will undoubtedly increase the number of examples. Aside from that, we have in Northern Patagonia and even more, the further south we go, an increasing number of species with a bipolar disjunction of area, species which are definitely identical with boreal species of the northern hemisphere and may as such be compared with the current example in phanerogamic botany: *Primula farinosa,* occurring both in the Patagonian Andes and in the mountains of the northern hemisphere. Such examples of fungi are *Omphalina griseopallida, Galerina vittaeformis, Inocybe eutheles, Clitocybe suaveolens, Melanoleuca excissa* and

M. evenosa, Hygrophoropsis aurantiaca, Hohenbuehelia petaloides, and in other cases we may indicate pairs of corresponding, extremely closely related species of the same stirps, one occurring in the boreal regions of the northern hemisphere, the other in the *Nothofagus* area: *Camarophyllus lacmus* as reported from Iceland, and *C. adonis* from Tierra del Fuego, *Pluteus atromarginatus* (Sing.) Kuehner and *P. spegazzinianus* from Tierra del Fuego, *Clitocybe leptoloma* from the United States and Canada, and *C. subleptoloma* from Tierra del Fuego, *Melanoleuca cognata* and *M. longispora, Phacomarasmius distans* and *P. chiliotrichi, Bondarzewia berkeleyi* and a new species of *Bondarzewia* on *Nothofagus dombeyi* in the Nahuel Huapi region, and finally *Leucopaxillus rhodoleucus* and *L. patagonicus.* The explanation of this disjunction is perhaps one of the most challenging problems of historical plant geography.

The mountain meadows within the *Nothofagus* area show a rich fungous flora containing *Armillariella pumila, Omphalina defibulata, Camarophyllus tehuelches, Agaricus pampeanus, Galerina clavata, Marasmiellus fibula;* the numerous *Sphagnum* bogs contain *Galerina riparia, Naematoloma myosotis;* the alpine zone has not been touched so far. Saprophytic species, characteristic of the woods are, among others: *Fistulina antarctica,* three species of *Lepista,* several species of *Clitocybe,* two species of the smooth-spored group of lignicolous species included in *Fayodia* by me, *Pluteus jaffuelii* (Speg.) Sing. (*Mycena jaffuelii* Speg.), one *Lepiota* of the *acutesquamosa* group, one new *Kuehneromyces, Crepidotus brunswickianus* and *C. nephrodes, Coprinus atramentarius* vel aff. and *C. comatus* v. aff., *Tubaria gregoriana,* many species of *Melanoleuca, Panellus longinquus,* species of *Clavariella, Glocosoma vitellinum.* Among the mycorrhiza-forming Agaricales: Several species of *Porpoloma, Russula, Cortinarius, Inocybe, Tricholoma, Amanita,* one *Hebeloma,* one *Descolea.* Among the Secotiaceae, we noticed *Thaxterogaster maghellanicus* (very common), and *T. violaceus* (always sessile), both formerly collected by R. Thaxter near Punta Arenas (Chile); among the Discomycetes we observed numerous Helotiaceae and Morchellae and *Gyromitra,* several species (at least three) of *Cyttaria.* The

number of polypores is not so large as in the tropical and subtropical regions, which is characteristic for the boreal-subarctic and for the subantarctic regions in general. There is, however, *Crinipellis ursina* (Speg.) Sing. and *Favolaschia antarctica* (Speg.) Sing. apparently growing on *Nothofagus;* there is furthermore *Marasmiellus couleu* growing on *Chusquea couleu,* and *Campanella dendrophora* Sing. & Dig. spec. nov. ined. on the same host. All of these, including the *Chusquea,* are species with northern relations, their respective genera having their most conspicuous development in the warmer regions of the continent. But these are exceptions. In general, the predominantly "tropical" genera are absent (*Nothopanus, Trogia, Pleurocollybia, Dictyopanus, Phaeomycena, Lactocollybia, Cymatella, Physalacria, Chaetocalathus, Hymenogloea, Heimiomyces, Filoboletus, Chlorophyllum, Leucocoprinus, Cystoagaricus, Melanophyllum, Smithiomyces, Ripartitella, Copelandia, Melanotus, Pleuroflammula, Pyrrhoglossum, Neopaxillus*) and even such genera as *Panus, Lentinus, Marasmius* were rarely encountered. More interesting, because, without reference to relative thermophily of the respective organisms, is the complete absence of representatives of the genus *Hygrophorus,* of *Alnicola,* of all Boletaceae (with the exception of *Boletus loyo*), all Strobilomycetaceae and Gomphidiaceae, the entire genus *Lactarius,* the genus *Hypsizygus* and *Pleurocybella, Tricholomopsis, Lyophyllum, Macrolepiota, Limacella,* all Cantharellaceae, etc., at least among the reported collections on the Chilean side, and on the entire Argentine side visited by me. Among the few genera that seem to have a particularly rich representation, so far as species as well as individuals are concerned, we name *Naucoria, Cortinarius* (and *Descolea*), and *Paxillus.* The large number of species of *Cortinarius* and related genera does not surprise those who understand the importance of the discovery of *Thaxterogaster,* the secotiaceous genus which seems to have been the starting point of this group of agarics (see Mycologia **43**: 215–228. 1951) but the variety in the Paxillaceae in southern South America in general, and in Patagonia in particular, is amazing. While it was thought that the presence of clamp connections is a family character in the Paxillaceae, we now find one species, *P. defibu-*

latus, without clamp connections, and we find two species of *Paxillus* with distinct veil (*Paxillus argentinus* from the Buenos Aires region, and *Paxillus boletinoides* from Patagonia-Tierra del Fuego).

Leaving the native woods and entering the extensive experimental plantations of conifers on Isla Victoria, Lago Nahuel Huapi, Neuquén, we find *Suillus luteus* conquering the entire pine area of the island as well as in nearby gardens and parks, regardless of the origin of the particular species. This, compared with the somewhat less exclusive but definitely dominant mycorrhiza of *Suillus granulatus* in the Rio de La Plata region, creates a new problem for forest mycologists interested in forestation. How can the mycologist help the forester in these problems? It seems obvious that the importance of the mycorrhizal partner at least in the nursery stage of the forestation project can hardly be exaggerated. It appears, then, that it is a further fact that the same pines, under different climatic and soil conditions, form mycorrhiza with different mycorrhizal partners. In order to establish the seedlings, it is consequently desirable to control the mycorrhizal association rather than to leave it to chance as is done at present. Another case which, in my opinion, shows that the exploration of the mycological flora of Southern South America is not merely a scientific necessity, is the discovery—not by a phytopathologist—of the above mentioned species of *Bondarzewia*,[5] a species highly damaging to the most valuable lumber resource of Patagonia. Its correct classification gives the forest pathologist, together with the observation, a hint where to look for analogous tree diseases (*Bondarzewia montana* on *Abies nordmaniana* in the Caucasus, *B. berkeleyi* on oak in North America). Another example is the observation of *Phaeogyroporus* in the area of intensive citrus cul-

[5] **Bondarzewia perniciosa** spec. nov. Pileo intense aurantiaco (pl. 4 A 12 vel "Pujab" Maerz & Paul), concentrice depresso, tomentoso, 140–160 mm. lato. Tubis dilute aurantiacis, breviusculis, irregularibus, decurrentibus. Stipitibus in truncum basalem dense concrescentibus carpophorum complexum multipileatum asque ad 1 m. latum formatibus. Carne tenacissima, aurantiaco-albida vel alba, admodum piperata. Sporis eis *B. montanea* (Quél.) Sing. similimis, amyloideo ornamentatis, subglobosis. Ad truncos vivos *Nothofagi dombeyi* arboresque necans. Typus Quetrihué, Neuquén, Argentina, Maio 1952, R. Singer M *608*.

ture in Argentina (Tucumán) where it causes the phytopathologist to watch for a dreaded disease reported in the Rio de Janeiro region by Brazilian phytopathologists, and connected with this bolete whose cryptas create the conditions under which the pathogenic organisms attack the roots. A similar threat is the presence in Patagonia, the Buenos Aires region, Brazil and Chile of either *Armillariella mellea* or a very closely related parasitic species, *A. puiggarii.*

Some of the species reported by us are edible and either now used by mushroom gatherers (the *Suillus luteus* of Isla Victoria is even the object of a concession given to a company in Bariloche for its exploitation in the National Park of Nahuel Huapi), or else of potential importance in the future. Most of the species of *Agaricus* growing in the pampa and mountain meadows belong in this category as only one plant producing canned white mushrooms—in mushroom houses in Martinez near Buenos Aires—operates at present. *Phaeogyroporus tropicus* is collected and dried for export into other provinces in the Sierras of Córdoba, and is used instead of imported *Boletus edulis* in oil (from Italy). Other edible species are: *Macrolepiota bonaerensis, Agrocybe aegerita, Armillariella mellea* and *puiggarii, Lepista diemii* (from Patagonia), *Boletus loyo* (from Patagonia, especially from Chile), *Mycenastrum corium* (here often called *M. chilense*) and other "puffballs," *Laetiporus sulphureus, Morchella.* We have established the edibility of *Marasmius pampicola, Pluteus spegazzinianus, Cantharellula tarnensis, Laccaria tetraspora.* Among the poisonous native fungi, we mention *Chlorophyllum molybdites, Amanita ameghinoi,* a little known species of *Volvariella,* and several species of *Inocybe,* probably also other species of *Amanita* found in the north and south of Argentina and in Chile, and a certain undetermined number of Lepiotas (in the widest sense).

The scientific significance of the material collected and described and the observations made in connection with this cannot be measured by the—perhaps impressive—number of new forms discovered. The only classification at hand applicable to the non-European Agaricales flora is that of the author (Lilloa 22, 1951), and we have always considered this system of classification

as being on trial by nature itself when it was attempted to insert the multitude of unknown species and of species formerly incompletely known into this new, or rather, partly new system. The fact that four years of most intensive research work in South America has unearthed only four new genera in the Agaricales and one in the Gastromycetes—and this without a large number of adjustments of the limits of the existing genera[6]—is taken as a favorable result as far as this system of classification is concerned. On the other hand, it was possible to add certain forms of taxonomic interest to the system, such as *Phylloboletellus*, the long-expected lamellate genus in the Strobilomycetaceae, *Gloiocephala* Mass. as re-collected and recharacterized (the *Marasmius* with smooth hymenophore), *Thaxterogaster* and *Brauniella*, the two new links between Gastromycetes and Agaricales from Tierra del Fuego and South Brazil respectively, the germporeless spe-

[6] These adjustments are so few that they can be enumerated here. The group of *Clitocybula*, inserted as a subgenus in *Fayodia* in my classification, contains Patagonian species with completely different habit. I do not hesitate to admit that this proves me wrong and Josserand and others who have insisted on the separation of this subgenus from the type subgenus (*Fayodia*, formerly *Eu-Fayodia*) were apparently right. It is proposed to consider *Clitocybula* a genus and transfer it to the *Leucopaxilleae*, in the neighborhood of *Cantharellula*. The genus *Asterotus*, thus far monomorphic, had also to be emended in order to accommodate a closely related but gelatinous species from Argentina. This suggests that *Asterotus* would be better placed in the neighborhood of *Resupinatus* than in its present position. A close observation of the forms of *Poromycena* in comparison with the section *Purae* of *Mycena* leads me to the conclusion that *Poromycena* is better considered a section or subsection of *Mycena*. A clearer understanding of Heim's proposed unit *Phacomycena* leads me to the conclusion that this latter is intermediate between *Lactocollybia* and *Callistosporium*, and both *Phacomycena* and *Lactocollybia* should be transferred to the neighborhood of *Callistosporium*. *Paxillus* must now be characterized as having clamp connections except for one species, and in *Panellus*, we must now remove the words "cystidia none," saying that cystidia do occur on the sides of the lamellae in some species of *Panellus*. By the discovery of more species in *Heimiomyces*, it appears now that this latter genus is closer to *Xeromphalina* than had been anticipated, even though the two were placed side by side in my classification. However, I do not think that, at the present time, there is any need of contemplating the generic identity of the two genera. But the number of new species inserted in *Armillariella* and *Marasmiellus* may make it possible to consider a division of each of these genera into two independent ones.

cies of *Galeropsis, G. allosperma* Sing., the species transitory between the section *Tubariopsis* and *Galerina* discovered in Brazil. Aside from that, certain genera which—although well known as such—consisted until now only of one species, were found to contain several species, and the delimitation from the neighboring genera was made more secure. A further interesting aspect was the fact that those groups which were thought to be characterized not merely by a set of correlated morphological characters but by their ability to form ectotropic mycorrhiza with certain forest trees, were consistently absent where the mycorrhiza partners were absent, a confirmation of their absolute dependency on these hosts that could have been obtained only in difficult and not always fully convincing experimental work with hundreds of species involved. This aspect of our results has been dealt with in a special communication given to the XIIth International Botanical Congress and now in print.

Some of our observations seem to indicate that the subtropical rain forest is not—as it may seem—an absolutely ideal habitat for the normal type of fleshy fungi. Such a habitat is much rather the humid part of the "pampa," or the northwest Patagonian woods. In the Selva Boliviano-Tucumana, there are two factors detrimental to the normal development of fleshy Basidiomycetes: (1) stronger competition by other soil organisms, Actinomycetes, Hyphomycetes, bacteria, with a tendency toward faster destruction by decay of the larger carpophores; (2) stronger and more rapid changes in humidity of the humus and the air than in both truly tropical and temperate forests. The result is a tendency toward smaller carpophores, or more leathery or gelatinous carpophores, or else a tendency toward potential abbreviation of the life cycle achieved by sclerotized gonotoconts, and elimination of basidiospore formation and dispersion, or else by elimination of the reduction division (and hence abolishment of hymenophores). An interesting example of a species that forms basidiospores only in a nearly primordial stage and later abandons basidium formation in favor of large vesiculose terminal cells of the subhymenium which eventually sclerotize, is *Marasmius spinosissimus* Sing. It is the first case of a single organism being

a true agaric at first, and forming something closely approaching a *Rhacophyllus* form toward the end of its development. It may therefore serve as proof of the basidiomycetous character of *Rhacophyllus*. This is no new statement. Indeed, it is currently assumed that *Rhacophyllus* is a form of an agaric, but this statement was based on nothing more than an analogy in the cytology of the bulbils and the basidium, and the similarity of the shape of the carpophores. An example of imperfect agaric fructification could be given by describing the formation of both arthrosporial and basidiosporial fructifications in species of an *Armillariella, A. ditopa,* also from near Tucumán. The same mycelium may form both imperfect and perfect carpophores, the former clavarioid, the latter clitocyboid; but one is separated and independent from the other.

A modern critical review of South American species also throws a different light on the relative prevalence of certain genera in the tropics and subtropics. Using statistics derived primarily from Saccardo, Sylloge, one would assume that the richest genera in species inhabiting the tropics are *Pleurotus, Panus,* and *Lentinus, Collybia, Marasmius, Omphalia,* and *Lepiota*. This is only true as far as the last genus is concerned, and to a certain degree for *Marasmius* if *Marasmiellus* is included, also to a certain extent for *Collybia*. *Pleurotus, Panus,* and *Lentinus* are poorer in species, due to the fact that an enormous amount of later synonyms have been described, and this again is due to the fact that many of the species have been simultaneously described in all three of these genera, and many in some additional genera. This, in its turn, was unavoidable because of the lack of a criterium as to what characters should be attributed to these genera.

During our past studies on the material collected, certain groups were somewhat neglected, not because of lack of material or observation but rather because of the policy that guided the entire plan of work in South America, which consisted in the belief that specialists of certain genera would be better equipped to master the quantities of material and notes than nonspecialists. This caused me a certain delay in the determination of some Hygrophoraceae, *Mycena,* Agaricaceae and Coprinaceae, *Cortinarius*

and *Rhodophyllus*. It is, however, not really of any great influence in the interpretation of our material as a whole, since, in the tropical-subtropical belt all these groups, but *Lepiota* in the wider sense, and *Agaricus* in the narrow sense (Agaricaceae), and in the south all but *Cortinarius*, are of no more than average importance as constituents of the basidiomycetous flora. The vast and seemingly indomitable genus *Lepiota* needs a special and careful study, careful especially because of the fact that many species of this genus have been described in other genera not even related to it, by both Rick and Spegazzini. The study of *Agaricus* will not merely bring some order into the multitude of South American forms of a very important genus; it will, beyond that, influence the development of *Agaricus* studies in the northern hemisphere, since even preliminary type studies show that some northern species occur here, and some of these have probably been described in Argentina or Brazil (Rick frequently described them in *Pholiotella*) before they were described in Europe or North America. *Agaricus* has also practical importance. For regions like Tucumán or Rio de Janeiro, it will be necessary to select a local or pantropical species, or anyhow, a species with a higher optimum growth temperature than observed in *Agaricus bisporus* (Lange) Sing., the commercially grown species. Furthermore, as I have pointed out, there are numerous indications which suggest that *Agaricus* plays some rôle in the fungus-ant symbiosis so characteristic for wide stretches of South America. A study similar to that made on the relationship of termites and *Termitomyces* in the palaeotropics should result in South America as soon as more is known about the genus *Agaricus*. There should be no difficulty in growing the fungus frequently collected by entomologists (who like to call their symbiotic ants "agriculturists") to fructification. Even if the perfect form of the species involved were not *Agaricus*, the latter must have at least an epibiotic relationship to the fungus-ant complex.

Geographically speaking, there is no major white spot left in the fungus map of Southern South America. Nevertheless, there are minor spots of interest. A more careful collecting program with the aim of establishing the relation between the mycoflora of

the Selva Boliviano-Tucumana and the Upper Amazonas should plan visits to Orán, the Cochabamba forests in Bolivia, and the eastern slope of the Andes in Perú. Collecting in the territory of Formosa and all the way down along the Paraná will undoubtedly open up certain unknown subfloras in and around the wetter part of the Chaco, and clear up its mycological relation with the Selva Misionera. A trip to Valdivia will round out our knowledge of the northern portion of the Patagonian *Nothofagus* area, and collecting in the subalpine and alpine regions of the Southern Cordillera might mean the opening up of an untouched field. This can easily be done by studying the mycoflora of relatively accessible places like the western part of the mountains around Lago Argentino. It is also hoped that hosts like *Fitzroya* (at the Laguna Frias or in Chile), and the study of llama and guanaco and *Hippocamelus* dung in remote places of South America might throw more light on the question as to whether any of the common coprophilous species are indigenous in this continent. As for the understanding of historical mycogeography in the great lines, the exploration of the continent should now be directed to its immense and practically untouched *terra incognita*, the Amazonas basin and the Upper Orinoco, the northwestern mountain range in Ecuador and Colombia with special attention to be paid to the boreal elements (if such exist) along the upper limits of vegetation and in the oak forests of Colombia. South American mycologists will also show great interest in the further exploration of the basidiomycetous flora of the *Nothofagus* area of New Zealand.

Even though our knowledge of the continent as a whole is sketchy, and perhaps not more than a quarter or one half of the species of southern South America has been mapped, it is now possible to say that a start has been made, that further work has been outlined and made easier by the existing type studies and the prodromus of an Argentine agaric flora and a survey of the South Brazilian basidiomycetous flora. This was possible thanks to the farsighted and inspired organization of biological research initiated and directed by the Director of the Instituto M. Lillo, Dr. Horacio R. Descole and, also, by the faithful and disinterested cooperation of the—unfortunately so few—botanists scattered all over southern

South America, especially B. Rambo, J. C. Lindquist, J. E. Wright, Espinosa, Diem, Cárdenas, and my colleagues at Tucumán. An acknowledgment is also due to the financial support given to the mycological work carried out by the Universidad Nacional de Tucumán, the Fundación Miguel Lillo, and the Guggenheim Memorial Foundation.

NEW SPECIES OF GALERINA FROM NORTH AMERICA [1, 2]

ALEXANDER H. SMITH

In the following account twenty-eight heretofore undescribed species of *Galerina* are treated. These were collected in the Great Lakes region and along the Pacific Coast during the period from 1935 to 1952. As a rule, species of *Galerina* are not a conspicuous element in our agaric flora, but during the warm dry season of 1952 they were found in abundance in Mount Rainier National Park. During the fall of 1952 no appreciable amount of rain fell in the Park from around the tenth of September until the end of October, and the forest floor, ordinarily covered during this period with fruiting bodies of many species in various genera of the fleshy fungi, was barren. Fungi were found only at high elevations and in local pockets where there was moisture and protection from the sun. One notable area of this sort was at Green Lake in the Carbon River district of the Park. The north-facing slopes of the Tatoosh Range furnished many favorable spots, such as that around Snow Lake.

Collecting was carried on in these areas as well as at higher elevations until long after the usual time for the fall snows and the advent of winter. Thus the 1952 season in the Park was truly unusual, and served well to illustrate the need for collecting in an area under all types of weather conditions before drawing many conclusions about either the diversity of a fungous flora or the fruiting pattern of the species comprising it. During the season of 1948, one of the wettest years in the history of the Park, the same areas produced practically nothing in the way of fungi during the summer and were covered by snow early in September.

[1] Papers from the University of Michigan Herbarium and the Department of Botany, University of Michigan, Ann Arbor, Mich.

[2] Published out of order as excess pagination at cost of University of Michigan Herbarium.

Kühner (1) monographed *Galerina* for France as part of a treatment of the Friesian genus *Galera*. In this work he emphasized spore structure as one of the important diagnostic characters. As he defined the genus, the smooth suprahilar depression or "plage" of the spore was the central character, and this has been followed by Singer (3). It is also followed in the present paper. Both earlier authors included entirely smooth-spored species in the genus, as I have done here, and a few in which the suprahilar area as well as the rest of the spore surface is roughened. However, the latter species are regarded as peripheral. Assignment to *Galerina,* in such instances, is of course made on the aggregate of all the other characters.

In his work Kühner treated eighteen species. I have accounted for practically all of these in North America; the twenty-eight additional species treated in this paper are only the more outstanding discoveries that we have made. In fact, when the study is complete, I predict that there will be around sixty species from North America. Thus *Galerina* is not a small genus, as some have thought.

Smith and Singer are at present engaged in a monographic study of the genus, and for this reason illustrations of carpophores, drawings of microscopic characters, and discussions of relationships and speciation are not presented here. These can all be treated more effectively in a work dealing with all known species.

The type collections are deposited in the University of Michigan Herbarium. In regard to species collected in our national parks, if the collections were large enough to be divided, a part has been deposited in the Mount Rainier National Park Herbarium. The color terms in quotation marks are taken from Ridgway.[3] The observations on the spores were made from dried specimens revived in 2.5 percent KOH, and viewed with a 1.3 NA oil-immersion objective and a 15 × eyepiece. This is important to anyone seeking to compare spores with the descriptions published here because the spore markings are exceedingly minute in some species.

[3] Ridgway, R. Color standards and color nomenclature. Washington. D. C. 1912.

The collecting expeditions on which these fungi were obtained were made possible largely by grants from the Faculty Research Fund of the University of Michigan during the years from 1935 to 1952, and in part by the generosity of Mr. Wm. B. Gruber of Portland, Oregon. The University of Michigan Herbarium also financed some of the field work during this period, and facilities for the collecting in northern Michigan were furnished by the University of Michigan Biological Station located on Douglas Lake.

I am particularly indebted to the National Park Service for its cooperation in furnishing facilities, equipment, and other aids upon a number of occasions. I wish, again, to express my personal appreciation to Superintendent Preston P. Macy and his staff for courtesies extended to me on my visits to the Olympic National Park in 1939 and 1941; to Mr. John C. Preston, then superintendent of Mount Rainier National Park, for courtesies extended to my party at Mount Rainier during the season of 1948. Superintendent Preston P. Macy, now of Mount Rainier National Park, and his staff were of great assistance during the dry season of 1952. It was through the guidance of members of the naturalist staff that the best localities were found and visited during 1952.

1. **Galerina atkinsoniana** sp. nov.

Pileus 3–5(–7) mm latus, 3–5 mm altus, conicus, fulvus vel pallide fulvus; lamellae distantes, subventricosae, late adnatae; stipes 2–3 cm longus, c. 1 mm crassus, aequalis, subpubescens; sporae 11–14 × 6.5–8 μ; pleurocystidia 38–65 × 10–15(–16) μ; pilocystidia pleurocystidiis similia. Specimen typicum in Herb. Univ. Mich. conservatum; legit prope Upper Falls, Tahquamenon Falls State Park, Mich., Aug. 23, 1951, Smith n. *38184*.

Pileus 3–5(–7) mm broad at base and 3–5 mm high, obtuse becoming campanulate to convex, glabrous (or pubescent under a lens), moist, hygrophanous, "russet" to "tawny," fading to "warm buff" (fulvous to tawny, fading to yellowish), striate to apex when moist; flesh very thin, watery-fragile, tawny to ochraceous, odor and taste not distinctive; lamellae broadly adnate, broad (1.5 mm), distant, subventricose, tawny at maturity, pale ochraceous when young, edges white-fimbriate and slightly eroded; stipe 2–3 cm long, about 1 mm thick, equal, fragile, lower part concolorous with or darker brown than pileus, paler above, or at first con-

colorous throughout, densely pruinose from projecting caulocystidia, no veil present on buttons.

Spores 11–14 × 6.5–8 μ, ovate-apiculate in face view, slightly inequilateral in side view, rugulose to minutely warty as revived in KOH, some showing 1–3 globules in the interior, a very minute apical pore present as seen under oil; basidia 2-spored, 27–34 × 7–8 μ, projecting slightly when sporulating; pleurocystidia scattered, fusoid-ventricose, 38–65 × 10–15(–16) μ, thin-walled and hyaline in KOH; cheilocystidia abundant to scattered, 28–46 × (8–)9–14(–17) μ, narrowly to broadly fusoid-ventricose, thin-walled, hyaline in KOH; gill trama subparallel to interwoven; cells short and relatively broad, pale ochraceous from incrusting pigment when revived in KOH; pileus trama homogeneous, pale ochraceous to tawny in KOH, pigment incrusted and more conspicuous (darker) toward cap surface, no appreciable pellicle differentiated, pilocystidia present and similar to the pleurocystidia, clamp connections present; caulocystidia abundant, hyaline, thin-walled, 60–120 × 9–18(–20) μ, fusoid-ventricose.

Habit, habitat, and distribution: Scattered among mosses on the ground or on old logs, apparently northern and not common. It is to be expected during the summer and early fall.

Observations: This species is characterized by the large pilocystidia resembling the pleurocystidia. Both 2-spored and 4-spored pilei have been found. The pruinosity on the stipe is caused by numerous caulocystidia. The collections referred here were first placed in *Galerula muricellospora* Atkinson, until a study of Atkinson's type revealed that it contained no carpophores with pilocystidia.

2. **Galerina camarinoides** sp. nov.

Pileus 4–7 mm latus, obtuse conicus, demum late conicus, pallide argillaceus; lamellae distantes, late adnatae, latae; stipes 10–15 mm longus, c. 1 mm crassus, pruinosus, dilute ochraceus; sporae 9–11 × 5–6 μ, pallide ochraceae; cheilocystidia 18–36 × (3–)4–8(–9) μ, ventricosa et capitata vel cylindrica et capitata. Specimen typicum in Herb. Univ. Mich. conservatum; legit prope East Fork, Salmon River, Mt. Hood, Ore., 3,800 ft. alt., Smith n. *19374*.

Pileus 4–7 mm broad, obtusely conic, the margin appressed against the stipe when young, becoming broadly conic, near cinnamon buff on disc, margin paler and near pinkish buff, translucent-striate, hygrophanous, becoming slightly paler and opaque when

faded, glabrous at all times; flesh very thin, concolorous with surface, odor and taste not distinctive; lamellae distant, 8–10 reach the stipe, 1 tier of lamellulae or none at all, broadly adnate, broad, pale ochraceous tawny, edges even; stipe 10–15 mm long, ± 1 mm thick, equal, fragile, pale honey color, coarsely pruinose clear to apex.

Spores 9–11 × 5–6 μ, subelliptic in side view, elliptic in face view, ochraceous tawny or somewhat darker in KOH, very minutely punctate-roughened with a smooth suprahilar depression, apical pore present; basidia two- and four-spored, 26–30 × 8–9 μ, hyaline in KOH; pleurocystidia none; cheilocystidia 18–36 × (3–) 4–8(–9) μ, cylindric-capitate to ventricose at base and capitate at apex, neck often very narrow (± 3 μ), hyaline and thin-walled in KOH; gill trama interwoven, pale yellow in KOH; pileus trama near surface tawny in KOH from heavy incrustations of pigment, clamp connections frequent; caulocystidia 30–62 × (+-)6–12 μ, variable in shape but the general pattern cylindric to subventricose with capitate apices.

Habit, habitat and distribution: Gregarious on moss over rock, East fork of Salmon River, Mt. Hood, Ore., 3800 ft. elev., Sm-*19374*.

Observations: The cheilocystidia are definitely the *G. sideroides* type but the spores, under a good oil immersion lens, are seen to be typical of *Galerina*. No veil was noted in my collection, but in Hesler *17642* a faint band of fibrils is visible on the stipe indicating veil remnants. This fungus cannot be *G. camerina* (Fr.) Kühner because the stipe is not "*umbrino*" as Fries described it.

3. **Galerina carbonicola** sp. nov.

Pileus 8–17 mm latus, conico-campanulatus, glaber, "russet" vel "tawny" (non latericius); lamellae adnatae, confertae, angustae; stipes 4–6(–8) cm longus, 1.5–2.5 mm crassus; deorsum subbadius; sporae 8–10.5 × 4.5–5.5 μ; cheilocystidia 35–45 × 9–12 μ, fusoidi-ventricosa; carbonicola. Specimen typicum in Herb. Univ. Mich. conservatum; legit prope Whitmore Lake, Mich., Sept. 28, 1933, Smith n. *33–1032*.

Pileus 8–17 mm broad, conic-campanulate to convex and with a small obtuse papilla, margin at first often curved in against gills, conic-campanulate, glabrous, hygrophanous, when moist "russet" or "tawny," when faded "ochraceous buff" (yellowish), striate nearly to apex, opaque when faded; flesh thin, fragile, watery-cinnamon brown when moist, yellowish when faded, odor and taste

not distinctive; lamellae adnate to slightly adnexed, close, rather narrow (± 2.5 mm) but finally somewhat ventricose near stipe, concolorous with pileus both when young and mature, edge minutely fimbriate; stipe 4–6(–8) cm long, 1.5–2.5 mm diam., concolorous with pileus or a little paler above and darker below, minutely pruinose above, scantily appressed-fibrillose below, some appearing longitudinally striate from appressed fibrils, base usually slightly bulbous to evenly enlarged.

Spores 8–10.5 × 4.5–5.5 μ, narrowly subinequilateral in side view, narrowly ovate in face view, russet in KOH at maturity, the outer layer present as irregular warts or deeply wrinkled, suprahilar depression smooth, apical pore present but indistinct; basidia four-spored, 24–28 × 7–8 μ, hyaline in KOH, projecting half their length when sporulating; pleourocystidia, none seen; cheilocystidia abundant, 35–45 × 9–12 μ, fusoid-ventricose, the necks often flexuous, the apices subacute to obtuse; gill trama more or less interwoven, rusty brown to yellow revived in KOH; pileus trama homogeneous, hyphae loosely interwoven and rusty brown from incrusting pigment, becoming pallid ochraceous on standing in KOH, no pellicle differentiated but hyphae of pellicular region radial and many narrower than those of tramal body; clamp connections present.

Habit, habitat and distribution: Under brushes on burned area, Mud Lake Bog, Whitmore Lake, Mich., Sept. 28, 1933, Sm-*33–1032*.

Observations: The russet to tawny color of pileus, gills and stipe, the rudimentary veil, the narrowly ovate spores in face view and the fusoid-ventricose cheilocystidia are distinctive, along with the carbonicolous habitat. Pleurocystidia similar to the cheilocystidia are to be found around portions of the hymenium damaged, possibly, by insects.

4. **Galerina cortinarioides** sp. nov.

Pileus 1–2 cm latus, late conicus, dilute fulvus, striatus; lamellae confertae, adnatae, latae, subfulvae; stipes 3–4 cm longus, 2–3.5 mm crassus, fragilis, deorsum demum subbadius, albofibrillosus, sursum albo-zonatus; sporae 8–11 × 6–7 μ; cheilocystidia 32–46(–55) × 6–10(–12) μ, fusoidi-ventricosa. Specimen typicum in Herb. Univ. Mich. conservatum; legit prope Green Lake, Mt. Rainier National Park, Wash., Sept. 18, 1952, Smith n. *40019*.

Pileus 1–2 cm broad, obtuse with a straight margin, expanding to broadly conic or margin spreading and disc a flattened umbo, surface glabrous except for a few marginal fibrils, hygrophanous,

"ochraceous tawny" or darker moist and then translucent-striate, ± cinnamon buff faded; flesh thickish but soft and fragile, watery ochraceous, taste slightly rancid (not farinaceous), odor none; lamellae broad, close, adnate, ventricose, pale to dark ochraceous tawny, edges crenulate; stipe 3-4 cm long, 2–3.5 mm thick (thickish as in *Cortinarius*), very fragile, pallid-brownish above, darker below and base bister in age, surface copiously decorated with remnants of a white veil but apical zone soon evanescent.

Spores 8–11 × 6–7 μ, ovate in face view, inequilateral in side view, ochraceous tawny, outer wall wrinkled, fitting like a sack over the spore and with loose ragged boundaries at hilar end, suprahilar depression smooth and marked by a ragged line or blisters of loosened exosporium; basidia four-spored, 23–30 × 8–9 μ; pleurocystidia none; cheilocystidia abundant, 32–46(–55) × 6–10 (–12) μ, fusoid-ventricose with long neck and obtuse apices, hyaline, or some with yellow content as revived in KOH, thin-walled; gill trama regular to subinterwoven, ochraceous in KOH from incrusting pigment; pileus trama with a thin pellicle of narrow (3–5 μ) nongelatinous hyphae with pale ochraceous-tawny incrusting pigment, tramal body of broader hyphae and also with incrusted pigment; clamp connections present.

Habit, habitat and distribution: Gregarious on soil in trail to Green Lake, Mt. Rainier National Park, Wash., Sept. 18, 1952, Sm-*40019*. Additional collections are Sm-*40097* and *40860* also from the Park.

Observation: In aspect this fungus resembles the small Cortinarii around *C. acutus* but the spores place it in *Galerina*.

5. **Galerina cuspidata** sp. nov.

Pileus 10–15 mm latus, 10–12 mm altus, cuspidatus, viscidus, dilute-cinnamomeus; lamellae confertae, latae, ventricosae, adnatae, pallide argillaceae; stipes 2–3 cm longus, 2–2.5 mm crassus, sparsim fibrillosus; sporae 8–9 × 4.5–5 μ (10–12 × 5–5.5 μ), leves; cheilocystidia 18–32 × 4–6.5 μ, anguste ventricosa et subcapitata. Specimen typicum in Herb. Univ. Mich. conservatum; legit prope Upper French Creek, Medicine Bow Mts., Wyo., Aug. 14, 1950, Nancy Jane Smith (A.H.S. n. *35716*).

Pileus 10–15 mm broad at base, 10–12 mm high, sharply conic to cuspidate, becoming conic campanulate, margin straight at first, surface viscid, glabrous or with flecks of fibrils from veil along the margin, "Sayal brown" (dull cinnamon) moist, hygrophanous, fading to yellowish or finally pallid, moist margin only faintly striatulate; flesh thin, pliant, concolorous with surface, odor and

taste none; lamellae close to crowded, broad and ventricose, adnate to adnexed but ascending, "pinkish buff" young, gradually becoming "cinnamon," edges even; stipe 2–3 cm long, 2–2.5 mm thick at apex, equal, pallid above, honey color below, base darker in age, surface thinly fibrillose from remains of pallid veil, apex naked to minutely pruinose.

Spore 8–9 × 4.5–5 μ (11–12 × 5–5.5 μ), smooth, no apical pore visible (under oil), ellipsoid, in side view the ventral line straighter than the dorsal; basidia four-spored (some 2-spored?); pleurocystidia none seen; cheilocystidia 18–32 × 4–6.5 μ, narrowly fusoid-ventricose to narrowly ventricose-subcapitate, often variously contorted; gill trama parallel, the subhymenium gelatinous; pileus trama cinnamon in KOH except for the gelatinous pellicle of narrow hyaline hyphae (3–5 μ); clamp connections present.

Habit, habitat and distribution: Gregarious on moss in a springy area, North Fork, French Creek, Medicine Bow, Mts., Wyo., Aug. 14, 1950, coll. by Nancy Jane Smith (A.H.S.-*35716*).

Observations: This species is related to *Galerina sideroides* but is easily distinguished in the field by the cuspidate pileus. The spores are consistently wider and usually longer than in *G. sideroides* and the gelatinous subhymenium is very distinct.

6. **Galerina farinosipes** sp. nov.

Pileus 1–2 cm latus, ovalis vel convexus, canescens, pallide fulvus; lamellae late adnatae, secedentes, confertae vel subdistantes; stipes 4–6 cm longus, 2–4 mm crassus, subtomentosus, deorsum fusco-brunneus; sporae 9–12 × 6–7 μ; pleurocystidia ventricose-subcapitata vel cylindrico-capitata, 44–56 × 9–12 μ; cheilocystidia 40–65 (–75) × 8–10 μ, cylindrica et capitata. Specimen typicum in Herb. Univ. Mich. conservatum: legit in terra prope Trinidad, Calif., Nov. 29, 1935, Smith n. *3649*.

Pileus 1–2 cm broad, oval to convex young, the margin straight, expanding to campanulate to broadly convex, surface at first conspicuously white-hoary from a dense coating of fine white fibrils, margin usually decorated with patches of the thin veil, glabrescent, surface moist, hygrophanous, "ochraceous tawny" and faintly striate to opaque on the margin, fading to near "warm buff"; flesh rather thick and firm, near ochraceous tawny and fading to buff, odor and taste not distinctive; lamellae moderately broad, adnate with a decurrent line, soon seceding, close to subdistant, pallid brown becoming ochraceous tawny, edges white floccose; stipe 4–6 cm long, 2–4 mm thick, equal, hollow, cartilaginous-fragile, usually somewhat twisted, densely pruinose-tomentose over all from a

dense grayish white tomentum, interior dark brown to bister in basal part, yellowish above.

Spores 9–12 × 6–7 μ, inequilateral in side view, ovate in face view, ochraceous tawny or paler in KOH, nearly smooth (as seen under oil minutely warty-rugulose), with a ragged line marking the suprahilar depression, apical pore if present very minute; basidia two-spored, 26–32 × 7–8 μ, hyaline; pleurocystidia scattered to abundant, 44–56 × 9–12 μ, ventricose-subcapitate to subcylindric-capitate or at least apices broadly rounded, hyaline, thin-walled; cheilocystidia abundant, 40–65(–75) × 8–10 μ, cylindric-subcapitate or the elongated part flexuous, hyaline and thin-walled; gill trama interwoven, yellowish in KOH; pileus trama homogeneous, yellowish in KOH, clamp connections present but rare, many cylindric-subcapitate pilocystidia arising from the cap surface, these cells 100–300 × 7–10 μ (these often appearing to form a nongelatinous pellicle).

Habit, habitat and distribution: Gregarious on soil, Trinidad, Calif., Nov. 29, 1935, coll. H. E. Parks (Smith-*3649*).

Observations: This species is grouped with *G. pruinatipes* because of the broadly rounded pleurocystidia and cheilocystidia and the presence of pilocystidia. Actually, however, the two species are quite different in appearance. *G. farinosipes* has a rather heavy sheath of white veil fibrils over the lower part of the stipe. The pilocystidia cause the white-hoary appearance of young caps. In age these elements are considerably elongated and matted down into a layer that can easily be mistaken for a pellicle if only cross sections of the gills and pileus are cut. The spores of *G. farinosipes* are scarcely roughened (as seen under oil) but the suprahilar depression is clearly bounded by a ragged line. The spores of *G. pruinatipes* are, relatively speaking, conspicuously roughened.

7. **Galerina ferruginea** sp. nov.

Pileus 8–12 mm latus, conicus demum late conicus vel campanulatus, interdum convexus, latericius; sapor farinaceus; lamellae latae, ventricosae, subdistantes, adnatae, secedentes; stipes 2–3.5 cm longus, 1–1.5 mm crassus, deorsum ferrugineus, sparsim fibrillosus; sporae 8–10(–11) × 4–5 μ; cheilocystidia 26–34(–46) × 6–10 μ, fusoidi-ventricosa et subcapitata; carbonicola. Specimen typicum in Herb. Mich. conservatum; legit prope Whitmore Lake, Mich., Sept. 25, 1933, Smith n. *33–1016*.

Pileus 8–12 broad at base, conic with a straight or connivent margin young, expanding to broadly conic, campanulate or convex

at maturity, glabrous, moist *"Hays russet"* to *"cinnamon rufous"* or *"ferruginous,"* subhygrophanous and reddish tan faded, when moist only faintly translucent-striate; flesh thin, concolorous with surface, *odor and taste slightly farinaceous;* lamellae broad, becoming ventricose, subdistant, adnate, seceding, pale brown at first, ochraceous orange at maturity, edges even to irregular; stipe 2–3.5 cm long, 1–1.5 mm thick, equal or enlarged above, tubular, lower half concolorous with pileus, upper portion paler, glabrous except for scattered fibrils over lower portion representing the remains of the rudimentary veil.

Spores 8–10(–11) × 4–5 μ, inequilateral in side view, ovate in face view, tawny or darker in KOH, minutely tuberculate, exospore loosening somewhat over suprahilar area but that area not entirely smooth, apical pore minute; basidia four spored, hyaline in KOH, 23–26 × 6–7 μ; pleurocystidia none; cheilocystidia abundant, 26–34(–46) × 7–10 μ, fusoid-ventricose with wavy necks and obtuse to capitate apices, thin-walled, hyaline in KOH; gill trama subparallel to interwoven, dull yellow (near ocher yellow) in KOH from incrusting pigment, gradually fading out to pallid, pileus trama homogeneous, yellow in KOH but fading out to pallid as in gill trama, clamp connections present.

Habit, habitat and distribution: Gregarious on burned peat, Mud Lake Bog, Whitmore Lake, Mich., Sept. 25, 1933, Sm-*33–1016.*

Observations: The curious feature of this species is the manner in which particles of the outer layer of the spore wall adhere over the suprahilar depression. As a result, the boundary of the depression varies greatly in position from spore to spore, and the plage also varies in size and degree of irregularity. The fact that there is a tendency for the "plage" area to become smooth is here considered as a valid reason for referring the fungus to *Galerina.* The truly ferruginous red color and farinaceous taste as well as the habitat aid in distinguishing the species in the field.

8. **Galerina fibrillosa** sp. nov.

Pileus 10–12 mm latus, plano-umbonatus, siccus, fibrillosus, obscure vinaceo-brunneus; lamellae latae, confertae, adnatae, obscure vinaceo-brunneae; stipes 1–2 cm longus, 1–1.5 mm crassus, peronato-fibrillosus fibrillae vinaceo-brunneae; sporae 10–14 × 5.5–6.8 μ; cheilocystidia 40–60 × 10–14 μ, ventricosa et subcapitata. Specimen typicum in Herb. Univ. Mich. conservatum; legit ad terram, Gobler's Knob, Mt. Rainier National Park, Wash., Oct. 12, 1952, Smith n. *40850.*

Pileus 10–12 mm broad, obtuse when young, expanding to plano-umbonate or umbo obsolete, surface dry and coarsely matted-fibrillose or in buttons hairy fibrillose, "vernona brown" to "snuff brown" (fibrils appearing coarse under a lens); flesh thin and soft, bister in KOH when fresh, no distinctive odor or taste; lamellae broad, close, ventricose in age, adnate, "Verona brown" young, and in age ± concolorous with the pileus, edges minutely fimbriate from cheilocystidia (use a lens); stipe 1–2 cm long, 1–1.5 mm at apex, slightly thicker downward, fragile, dull brownish beneath a hairy-fibrillose coating similar to that on pileus and covering the lower two thirds, apex pruinose in age and lower portion ± glabrescent.

Spores dull ferruginous in deposits, dull cinnamon in KOH, 10–14 × 5.5–6.8 μ, inequilateral in side view, in face view narrowly ovate, smooth except for a rough border around the supra-hilar depression; basidia four-spored; pleurocystidia none; cheilocystidia 40–60 × 10–14 μ fusoid-ventricose, often with wavy necks and obtuse to slightly enlarged apices; gill trama somewhat interwoven, dingy cinnamon in KOH; pileus trama with a cuticle of loosely arranged crooked hyphae heavily banded with dull cinnamon, incrusted pigment, tissue beneath this layer paler cinnamon and more compactly interwoven; clamp connections present.

Habit, habitat and distribution: Gregarious on wet earth, Gobler's Knob, Mt. Rainier National Park, Wash., Oct. 12, 1952, Sm-*40850*.

Observations: This species is most closely related to *G. insignis* but differs in its darker color, more coarsely matted-fibrillose pileus, the darker gills, and the more well developed dark brown fibrillose covering of the stipe. The spores and cheilocystidia are somewhat similar in both. The fruiting bodies are not at all "*Galerina*-like" in appearance, but the rough boundary of the suprahilar depression would seem to exclude *Phaeomarasmius*, where they would otherwise go.

9. **Galerina fuscobrunnea** sp. nov.

Pileus 5–10 latus, conicus, fusco-brunneus; lamellae subdistantes, latae, adnatae; stipes 2–3 cm longus, 1 mm crassus, fusco-brunneus; sporae 7.5–9.5(–10.4) × 4.5–5.5 μ, anguste inaequilateralis; cheilocystidia 32–48 × 5–8 μ, anguste clavata, sparsim incrustata. Specimen typicum in Herb. Univ. Mich. conservatum; legit ad terram in paludibus, prope Waupantia Summit, Cascade Mts., Ore., 3500 ft. alt., Sept. 24, 1946, Smith n. *23701*.

Pileus 5–10 mm broad, conic with a straight margin, expanding to conic-campanulate, glabrous, moist, hygrophanous, disc near "mummy brown" at first, "buckthorn brown" and striate over marginal area when mature, fading to pale buff on disc first; flesh concolorous with surface, soft, odor and taste mild; lamellae broadly adnate, subdistant, broad, 1–2 tiers of lamellulae, pale yellowish brown (± honey color); stipe 2–3 cm long, 1 mm thick, tubular, equal, concolorous with cap or a shade paler and with a few inconspicuous fibrils indicating presence of a thin veil.

Spores 7.5–9.5(–10.4) × 4.5–5.5 μ, narrowly subelliptic to very slightly inequilateral in side view, *narrowly* ovate in face view, smooth to very minutely roughened (exospore not separating from endospore), tawny or darker in KOH, suprahilar plate smooth but very indistinct and faintly bounded as seen under oil, apical pore not visible; basidia four-spored, hyaline in KOH; pleurocystidia none; cheilocystidia abundant, 32–48 × 5–8 μ, narrowly clavate to subcylindric, often with flexuous walls, apices of some with slight incrustation, hyaline in KOH, thin-walled; gill trama interwoven, rusty brown in KOH; pileus trama homogeneous, rusty brown in KOH, clamp connections present.

Habit, habitat and distribution: Gregarious on humus under alder, swampy place below Waupanitia Summit, Cascade Mts., Ore., 3500 ft., Sept. 24, 1946, Sm-*23701*.

Observations: The narrow, practically smooth, spores are distinctive along with the cheilocystidia. The habitat in alpine swamps may also be significant along with the very dark brown color of young carpophores.

10. **Galerina humicola** sp. nov.

Pileus 10–15 mm latus, conico-campanulatus, demum plano-umbonatus, glabrescens, cinnamomeo-brunneus; lamellae confertae, adnatae, secedentes, latae; stipes 1.5–2 cm longus, c. 1 mm crassus, deorsum demum badio-umbrinus, sursum fibrilloso-annulatus; annulus albus; sporae 5.5–6.3 × 4–4.5 μ; cheilocystidia 32–48 × 5.8 μ, ventricosa et subcapitata. Specimen typicum in Herb. Univ. Mich. conservatum; legit ad terram prope Milford, Mich., June 5, 1951, Smith n. *15552*.

Pileus 10–15 mm broad, conic-campanulate, the margin only slightly incurved at first and soon straight, expanding to plane with a sharp conic umbo, surface moist, at first with scattered fibrils from the remains of the veil, soon glabrous, translucent-striate, "cinnamon brown" to "ochraceous tawny" at first, hygrophanous and "warm buff" (yellow) faded, fading on disc first;

flesh thin, concolorous with surface, very soft and brittle, odor and taste slightly pungent; lamellae close (15–18 reach the stipe), 2 tiers of lamellulae, adnate but seceding readily, broad (± 2 mm), dull ochraceous tawny over all, edges even; stipe 1.5–2 cm long, ± 1 mm thick, slightly enlarged at apex, concolorous with pileus at first but soon becoming dark sordid brown from the base upward, *with a superior whitish fibrillose zone from the remains of the veil,* zone evanescent, sparsely white-fibrillose toward the base.

Spores 5.5–6.3 × 4–4.5 μ, inequilateral in side view, in face view subelliptic to ovate, tawny to pale russet in KOH, exospore wrinkled to rugulose-tuberculate, suprahilar depression present and apparently nearly smooth, apical pore present but minute; basidia four-spored; 16–19 × 5–6.5 μ, hyaline in KOH; pleurocystidia none seen; cheilocystidia 32–48 × 5–8 μ, abundant, fusoid-ventricose to almost tibiform (narrowly ventricose with subcapitate apices), hyaline, thin-walled (a few with yellowish content as revived in KOH); gill trama interwoven to subparallel, dull yellow-brown as revived in KOH; pileus trama homogeneous, no pellicle, dull yellow brown in KOH from incrusting pigment; clamp connections present at base of basidium.

Habit, habitat and distribution: Subcespitose on black muck near a stump, Milford, Michigan, June 5, 1941, Sm-*15552*.

Observations: This species differs from *G. latifolia* in having an annular zone of veil fibrils, spores which lack blisters of the loosened outer layer, and in its habitat on muck instead of oak wood. I at first considered describing it as a variety of *G. triscopa,* thinking that in *G. triscopa, G. laticeps* and *G. humicola* we had a situation comparable to that of the varieties in *G. badipes.* However, *G. laticeps,* on the basis of what we know so far, is distinct on spore characters and hence does not fit into such a series. As for *G. humicola,* we have no intergrading series to connect it with *G. triscopa* in the characters mentioned, and in addition its spores appear to be shorter *and* broader than those of *G. triscopa.*

11. **Galerina insignis** sp. nov.

Pileus 8–25 mm latus, late conicus, siccus, fibrillosus, pallide argillaceus; lamellae latae, confertae, cinnamomeae; stipes 2–3.5 cm longus, 1–1.5 mm crassus, deorsum brunnescens, peronato-fibrillosus; fibrae argillaceae; sporae 12–15 × 5–6.3 μ; cheilocystidia 36–48 × 8–11 μ, ventricosa et subcapitata. Specimen typicum in Herb. Univ. Mich. conservatum; legit prope Green Lake, Mt. Rainier National Park, Wash., Oct. 17, 1952, Smith n. *41067.*

Pileus 8–25 mm broad, obtusely conic expanding to broadly conic or margin spreading and umbo obtuse, surface dry and fibrillose, near "cinnamon buff," in age paler and near pinkish buff; flesh moist, *exceedingly* fragile, odor slight, taste slightly of raw cucumber; lamellae broad, ascending adnate, close, pinkish buff young, "Sayal brown" (cinnamon) at maturity, edges fimbriate; stipe 2–3.5 cm long, 1–1.5 mm thick, equal, lower part copiously fibrillose with "cinnamon buff" fibrils, pallid and silky to pruinose above, ground color brownish, base darker in age on some specimens.

Spores 12–15 × 5–6.3 μ, narrowly oblong in face view, obscurely oblong-inequilateral in side view, dull cinnamon in KOH; basidia four-spored; pleurocystidia scattered to rare, 40–56 × 10–13 μ, fusoid-ventricose with obtuse to slightly enlarged apices, hyaline, thin-walled, smooth; cheilocystidia mostly ventricose-capitate, 36–48 × 8–11 μ, head often as broad as the ventricose part; gill trama interwoven, ochraceous brown from encrusted pigment (darker in KOH); pileus trama of two ± equal parts, the upper of loosely arranged, widely spaced, heavily encrusted (dark cinnamon) hyphae 5–10 μ in diam., lower portion compactly interwoven and more ochraceous; clamp connections present.

Habit, habitat and distribution: Gregarious on soil in an old pile of debris from an avalanche, Green Lake, Mt. Rainier National Park, Wash., Oct. 17, 1952, Sm-*41067*.

Observations: The spores and cystidia are those of a *Galerina,* but the duplex structure of the pileus is not typical of any genus known to me. The suprahilar depression is seen to be marked off faintly when viewed under a good oil immersion lens, but the outer spore layer is practically smooth also. In optical section the subapical thickening of the outer wall gives the impression of a pair of earmuffs. The thickening extends clear around the spore, as can be perceived by noting the slightly darker color-band in this region.

12. **Galerina lacustris** sp. nov.

Pileus 10–15 mm latus, campanulatus, glaber, subviscidus, nitens, fulvus; lamellae latae, confertae vel subdistantes, adnatae, pallide argillaceae; stipes 4–6 cm. longus, 2–3 mm crassus, mollis, flexuosus, sparsim fibrillosus; sporae 10–12.5 × 5–6.3 μ; cheilocystidia 38–60 × 8–11 μ, clavata vel subventricosa et subcapitata, sparsim incrustata. Specimen typicum in Herb. Univ. Mich. conservatum: legit in paludibus, Lake George, Mt. Rainier National Park, Wash., Oct. 12, 1952, Smith n. *40848*.

Pileus 10–15 mm broad, obtusely conic, expanding to broadly campanulate, at times umbo obsolete and cap convex, surface glabrous, *lubricous* to *viscid* to touch when wet, *shining,* striate to disc, moist, bright "ochraceous tawny," disc a little darker; flesh soft and fragile (but fleshy for the genus), concolorous with surface, odor and taste mild; lamellae *broad,* close to subdistant, broadly adnate and readily seceding, "cinnamon buff" at maturity (paler than cap and duller), paler when young, edges even; stipe 4–6 cm long, 2–3 mm at apex, equal, *very soft and fragile,* hollow, at first with a few faint appresed fibrils below, apex faintly pruinose, concolorous with margin of cap over all or in some apex a little paler but no true color change present, *often translucent* and *undulating.*

Spores 10–12.5 × 5–6.3 μ, elongate-inequilateral in side view, narrowly ovate in face view, smooth except for a ragged line around the depression, *ochraceous tawny* in KOH; basidia four-spored; pleurocystidia none seen; cheilocystidia abundant, 38–60 × 8–11 μ, mostly clavate with flexuous pedicels but some ± ventricose at base and some ± filamentose, apical enlargement often oval, often somewhat incrusted beneath the apical enlargement; gill trama somewhat interwoven, ochraceous in KOH; pileus trama with a thin poorly formed subgelatinous pellicle (in KOH), main tramal body ochraceous tawny and homogeneous (in KOH); clamp connections present.

Habit, habitat and distribution: Scattered among mosses and sedges at low water level of Lake George, Mt. Rainier National Park, Wash., Oct. 12, 1952, Sm-*40848.*

Observations: The incrustation over the upper part of the necks of the cheilocystidia is peculiar in this genus. The line marking the suprahilar depression of the spore is very faint but can be observed on most properly oriented spores.

13. **Galerina laticeps** sp. nov.

Pileus 6–12 mm latus, convexus, dilute cinnamomeus, amarus; lamellae confertae, 1.5–2 mm latae, crassiusculae; stipes 1.2–5 cm longus, 1.5–2 mm crassus, aequalis, cavus, dilute cinnamomeus; sporae 7–8 × 5–6 μ; cheilocystidia 30–46 × 7–9 μ. Specimen typicum in Herb. Univ. Mich. conservatum; legit in ligno Pseudotsugae, Rhododendron, Ore., Nov. 17, 1944, Smith n. *19865.*

Pileus 6–12 mm broad, convex to obtusely conic at first, broadly convex in age, margin apparently straight at first, surface glabrous, moist, hygrophanous, with a very faint fringe of fibrils along the

margin when young, evenly "Sayal brown" and only faintly striate in age before fading, fading to sordid cinnamon buff or paler; flesh thin, brittle, concolor, odor none, taste bitter; lamellae close to crowded, 1–2 tiers, depressed to rounded adnate, moderately broad, ventricose (1.5–2 mm), thickish, faces concolorous with cap, edges whitish; stipe 1–2.5 cm long, 1.5–2 mm thick, equal, hollow, concolorous with or slightly paler than the pileus, not darkening below, at first with a faint fringe of white fibrils from the remains of a thin veil, soon glabrescent, base not conspicuously mycelioid.

Spores 7–8 × 5–6 μ, ovate to almost angular-ovate in face view, in side view inequilateral, with a smooth suprahilar depression, endospore thick and tawny to near russet in KOH, outer layer loosely enveloping the endospore except for suprahilar depression and very wrinkled to rugulose as well as somewhat deciduous (if mount is crushed in KOH), apical pore indistinct under oil; basidia four-spored, hyaline in KOH; pleurocystidia lacking; cheilocystidia scattered, 30–46 × 7–9μ, thin-walled, hyaline in KOH, slightly ventricose at base, with a narrow neck and an obtuse to subcapitate apex, or filamentous-subcapitate; gill trama homogeneous, rather dark ochraceous tawny from incrusting pigment; clamp connections present—but most appear to be false clamps with no fusion of the clamp with the main hypha; pilocystidia similar to cheilocystidia, scattered to rare.

Habit, habitat and distribution: Gregarious on Douglas Fir log, Rhododendron, Oregon, Nov. 17, 1944, Sm-*19865* and *20143*.

Observations: The obtuse to convex pileus and relatively pale cinnamon pileus distinguishes this species from *G. triscopa* in the field, and the broader spores (almost angular-ovate in face view), distinguish it in the herbarium. A somewhat similar fungus occurs on hardwood in which pleurocystidia are present and in which a veil is absent. However, in it the distribution of pleurocystidia was quite erratic and more collections are needed to clarify the situation.

14. **Galerina occidentalis** sp. nov.

Pileus 10–18 mm latus, convexus, fulvus, *amarus;* lamellae confertae, angustae, cinnamomeae; stipes 2–3 cm longus, 2.5 mm crassus, subaequalis, fulvus, deorsum demum badius, sursum subannulatus; sporae 9–12 × 5.5–6.3 μ; cheilocystidia 26–34 × 6–10 × ± 2 × 3–4 μ, ventricosa et subcapitata. Specimen typicum in Herb. Univ. Mich. conservatum; ad terram, prope

Carbon River Entrance, Mt. Rainier National Park, Wash., Oct. 2, 1952, Smith n. *40441*.

Pileus 10–18 mm broad, obtuse to convex, expanding to plane or with a slight umbo, surface glabrous, moist, hygrophanous, *dark rusty cinnamon* to near tawny moist, fading to tan, striate before fading, fading to dingy cinnamon; flesh thin, concolorous with surface; taste *disagreeable-rancid;* lamellae horizontal, close, adnate, narrow to moderately broad, cinnamon, edges even to slightly fimbriate; stipe 2–3 cm long, up to 2.5 mm thick at apex, equal or enlarged downward, concolorous with or slightly paler than pileus, darkening below in drying, annulate above with a zone of fibrils, apex pruinose, lower portion sheathed with a thin white fibrillose coating, annulus at times evanescent.

Spores (8.5–)9–12 × 5.5–6.3 μ, ovate in face view, slightly inequilateral in side view, smooth or very indistinctly roughened, the depression smooth but boundary faint, tawny in KOH; basidia 2-spored; pleurocystidia none; cheilocystidia 6–10 μ broad at base and 26–34 μ long, *neck* ± 2 μ *thick* and often ending in a small capitelum (3–4 μ) with an apical thickening of the wall; gill trama parallel, cells enlarged and dark cinnamon in KOH; pileus trama homogeneous and dark cinnamon beneath a paler nongelatinous pellicle of narrower (4–6 μ) hyphae; clamp connections present.

Habit, habitat and distribution: On humus, Carbon River Entrance, Mt. Rainier National Park, Oct. 2, 1952, Sm-*40441*-type. An earlier collection, Sm-*30035*, was made in the Park Aug. 9, 1948 on wood at Lower Tahoma Creek.

Observations: *G. occidentalis* is closely related to *G. tahquamenonensis* but can be distinguished in the field by its very disagreeable taste and close, narrow lamellae. The variation in the habitat from lignicolous to "terrestrial" in *G. occidentalis* is not likely to mean much because of the great amount of lignicolous material in the soil in this area. The dark color of the pileus and gill trama, as well as their structure, is typical of *Psilocybe* but the spores preclude assignment to that genus. The simple structure of the cuticle of the pileus prevents *G. occidentalis* from being placed in *Alnicola*—where the spore characters of color and markings would be aberrant. The cheilocystidia, however, are reminiscent of that genus. Under the best oil immersion lens available it was clear that the pattern of markings on the spores was typical of *Galerina*.

15. **Galerina odora** sp. nov.

Pileus 1–3 cm latus, late conicus, glaber, subfulvus, odoratus (subfarinaceus); lamellae latae, confertae, adnatae, pallide argillaceae; stipes 3–6 cm longus, 2–3.5 mm crassus, deorsum sparsim fibrilosus; sporae 10–14 × 6.5–8 μ; cheilocystidia 36–50 × 7–11 μ. Specimen typicum in Herb. Univ. Mich. conservatum; legit St. Andrews Creek, Mt. Rainier National Park, Wash., Sept. 24, 1952, Smith n. *40210*.

Pileus 1–3 cm broad, obtusely conic, expanding to broadly conic or somewhat umbonate, surface glabrous, moist and hygrophanous, faintly striate, dark ochraceous tawny moist, fading to cinnamon buff; flesh buff color, thick in the disc, thin over margin, odor resembling that of fresh green corn, taste mild; lamellae broad, close, adnate, cinnamon-buff becoming ochraceous tawny or darker, edges white fimbriate; stipe 3–6 cm long, 2–3.5 mm at apex, evenly enlarged downward, lower part coated with appressed pallid fibrils or these in zones or patches, apex pruinose, brownish downward but when dried evenly reddish brown overall.

Spores 10–14 × 6.5–8 μ, ovate in face view, in side view inequilateral, with blisters of the loosened exosporium especially around the smooth suprahilar depression; basidia four-spored, 24–30 × 9–10 μ, with globular content; pleurocystidia none; cheilocystidia abundant, 36–50 × 7–11 μ, narrowly ventricose with obtuse to slightly enlarged apices or in some apex tapered rather abruptly; gill trama regular, cells greatly enlarged, pale ochraceous in KOH; subhymenium appearing cellular in sections; pileus trama with a thin pellicle of narrow, ochraceous, non-gelatinous hyphae, tissue beneath of broad, interwoven ochraceous hyphae; clamp connections present.

Habit, habitat and distribution: Gregarious on a creek bank, St. Andrews Creek, Mt. Rainier National Park, Wash., Sept. 24, 1952, Sm-*40210*.

Observations: *G. odora* is distinct from *G. cortinarioides* by virtue of the odor of fresh green corn when the flesh is crushed and by the larger spores. Like *G. cortinarioides, G. odora* is almost certain to be confused in the field with the small slender species of *Cortinarius*.

16. **Galerina olympiana** sp. nov.

Pileus 5–10 mm latus, convexus, margine primo involuto, demum planus, glaber; lamellae confertae vel subdistantes, angustae, crassiusculae; stipes 1–2 cm longus, c. 1 mm crassus, flexuosus, deorsum badius et luteo-fibrillosus; sporae 8–10 × 4.5–5.5 μ (10–12 × 6–7 μ); basidia tetraspora vel bispora;

pleurocystidia 35–56(–60) × 12–15(–20) μ, apice late rotunda. Specimen typicum in Herb. Univ. Mich. conservatum: legit in ligno *Populi trichocarpi*. Hoh River, Olympic National Park, Wash., June 6, 1939, Smith n. *14116*.

Pileus 5–10 mm broad, convex with a slightly incurved margin, expanding to plane or nearly so, surface glabrous, hygrophanous, when fresh and moist "tawny" and opaque, becoming "ochraceous tawny" and conspicuously striate before fading to "warm buff" (pale yellow) or paler; flesh thick (for such a small fungus) and firm, concolorous with surface, odor and taste not distinctive; lamellae subdistant to close (13–15), narrow, horizontally adnate or at times slightly toothed, *rather thick*, concolorous with pileus and pruinose; stipe 1–2 cm long, about 1 mm thick, equal, crooked to undulating, with a narrow tubule, concolorous with pileus or darker reddish brown below, apex coarsely pruinose, lower portion with scattered patches of appressed *yellowish fibrils*.

Spores 8–10 × 4.5–5.5 μ (10–12 × 6–7 μ, 2-spored) ovate in face view, subinequilateral in side view, tawny as revived in KOH, pale yellow fresh, slightly rugulose-roughened, apical pore present but apex not truncate, suprahilar depression both slight and smooth; basidia 2- or 4-spored, 17–20 × 7–7.8 μ, clavate, slightly projecting; pleurocystidia abundant, 35–56(–60) × 12–15(–20) μ, thin-walled, hyaline or in some the content yellow when fresh, apices broadly rounded, lower part ventricose, pedicel slender and elongated; cheilocystidia occasionally with a globular hyaline inclusion, forming a sterile band on gill edge, more nearly cylindric than the pleurocystidia, apices broadly rounded, 38–56 × 9–15 μ; gill trama parallel, tawny in KOH from heavily incrusting pigment, clamp connections present; pileus trama tawny to russet in KOH, the incrusting pigment heavy, homogeneous, pellicle not differentiated, occasional pilocystidia similar to pleurocystidia present.

Habit, habitat and distribution: Gregarious on wood of *Populus trichocarpa*, Hoh River, Olympic National Park, Wash., June 6, 1939, Sm-*14116*.

Observations: Small metuloid cysts 6–8 μ broad and of variable length are also present in the hymenium. These do not appear to be typical gloeocystidia, but may be an additional distinctive feature of this very unusual little agaric. They are rare, and, since there is a strong tendency for both basidia and cystidia to have colored or granular-refractive content, these may simply be basidioles in a certain stage of development.

The distinguishing field characters are the small size, habitat on

cottonwood, thickish gills, and yellowish veil fibrils. Microscopically the pleurocystidia are most like those of *G. pruinatipes* but the markings on the spores are faint—as in *G. farinosipes*. In addition, there is more of a tendency for the cystidia to have a colored content than in either of the others. There are a large number of hyaline fusoid-ventricose caulocystidia (with obtuse apices) mixed with the capitate caulocystidia. *G. pruinatipes* var. *fulvipes* is a robust fungus. Both *G. pruinatipes* var. *pruinatipes* and var. *fulvipes* are specifically distinct from *G. olympiana* by the markings on the spores.

17. **Galerina pallidispora** sp. nov.

Pileus 10–25 mm latus, campanulatus, "russet" demum fulvus; lamellae adnatae, confertae, latae; stipes 3–4 cm longus, 2–3 mm crassus, ± aequalis, "russet" vel fulvus, concolor, glaber, sursum pruinosus; sporae 9–13 × 5–6.5 μ, pallide ochraceae; cheilocystidia 24–32 × 6–9 μ, ventricosa et capitata. Specimen typicum in Herb. Mich. conservatum; legit in ligno Alni, Graves Creek, Olympic National Park, Wash., May 23, 1939, Smith n. *13694*.

Pileus 10–25 mm broad, obtusely conic expanding to campanulate, surface glabrous, moist, hygrophanous, "russet" at first but becoming "ochraceous tawny" before fading, "warm buff" faded and when dried pale buff; lamellae bluntly adnate, close, moderately broad, ochraceous tawny, edges even; stipe 3–4 cm long, 2–3 mm thick, equal or slightly enlarged downward, concolorous with cap and fading like it, not darkening in age or on drying, pruinose above, glabrous below except for mycelium around base.

Spores 9–13 × 5–6.5 μ, subinequilateral to nearly oblong in side view, narrowly ovate to suboblong in face view, *pale ochraceous* in KOH, surface minutely rugulose-warty and suprahilar plate faintly delimited, apical pore minute; basidia two-spored, 24–28 × 6–7.5 μ, hyaline; pleurocystidia none seen; cheilocystidia ventricose-capitate to ventricose with narrow necks, apices of some slightly incrusted or with mucilage cap, 24–32 × 6–9 μ, thin-walled, hyaline; gill trama parallel or nearly so, tawny to yellow in KOH; subhymenium not distinctive; pileus trama homogeneous, clamp connections present.

Habit, habitat and distribution: Scattered on alder logs, Graves Creek, Olympic National Park, Wash., May 23, 1939, Sm-*13694*.

Observations: The pale ochraceous spores, lack of pleurocystidia, small cheilocystidia (many with capitate apices), russet pileus and unicolorous stipe distinguish this species. The cheilocystidia and

aspect of the carpophore resemble those of *G. sideroides*, but the slightly roughened spores do not allow it to be placed in that group.

18. **Galerina pruinatipes** sp. nov.

Pileus 10–20 mm latus, obtusus vel convexus, demum planus, fiulvo-cinnamomeus, striatus, fragilis, amarus; lamellae latae, confertae, adnatae, secedentes, acie albido-fimbriatae; stipes 1–3 cm longus, c. 1 mm crassus, fragilis, deorsum badius, sursum pruinosus; sporae 10–12.5 × 6.3–8 μ; pleurocystidia 40–55 × 10–18 μ, ventricosa, capitata vel subcapitata; pilocystidia cheilocystidia similia; cheilocystidia 36–44 × 9–13 μ, cylindrica et capitata. Specimen typicum in Herb. Univ. of Mich. conservatum; legit in ligno coniferarum Green Lake, Mt. Rainier National Park, Wash., Oct. 17, 1952, Smith n. *41029*.

Pileus 10–15(–20) mm broad, obtuse, expanding to plane, surface glabrous, moist, hygrophanous, rich tawny fading to pinkish buff (±), translucent striate moist; flesh very delicate and fragile, odor none, taste bitter; lamellae close, broad, bluntly adnate and readily seceding, pale ochraceous tawny young, between cinnamon buff and "cinnamon" mature, edges crenulate; stipe 1–3 cm long, ± 1 mm thick, equal except for slight bulb at base, fragile, *dark* reddish brown below, pallid and coarsely pruinose above, with *scattered pallid fibrils* downward, *base with a slight tawny pubescence*.

Spores 10–12.5 × 6.3–8 μ, inequilateral in side view, in face view ovate, warty-roughened and outer layer separable around the smooth depression, near russet in KOH; basidia two-spored; pleurocystidia scattered, 40–55 × 10–18 μ, ventricose with broadly rounded to capitate apices, hyaline, thin-walled; cheilocystidia 36–44 × 9–13 μ, mostly cylindric-capitate but some fusoid-ventricose elements present; gill trama of enlarged hyphal cells ± ochraceous in KOH, subregular; pileus trama homogeneous, elements mostly enlarged but some hyphae 5–10 μ in diam. present over surface, pigment incrusted; clamp connections present; pilocystidia similar to cheilocystidia or with somewhat colored walls; caulocystidia similar to cheilocystidia but in general more variable in shape, dull cinnamon to hyaline in KOH, many projecting filaments also present.

Habit, habitat and distribution: Scattered on rotten wet conifer logs in cold places, Green Lake, Mt. Rainier National Park, Wash., Oct. 17, 1952, Smith n. *41029*.

Observations: The broadly rounded to capitate pleurocystidia, cheilocystidia, pilocystidia and caulocystidia along with the dark-

colored, rough spores with their conspicuous suprahilar depression are distinctive. The stipe as revived in KOH becomes very dark. There is considerable variation in the cheilocystidia. Some are fusoid-ventricose with merely obtuse apices whereas the other extreme is almost cylindric capitate. All those observed were hyaline or at the most only faintly ochraceous at the very base. The outstanding field characters are the obtuse to plane pileus, coarsely pruinose stipe over upper half, pallid fibrils over lower half, the darkening of the stipe from the base up, the *tawny mycelium matted* around the base, and the bitter taste.

GALERINA PRUINATIPES var. **fulvipes** var. nov.

Pileus 1.5–3 cm latus, obtuse conicus demum subcampanulatus vel planoumbonatus; stipes 5–6 cm longus, c. 2 mm crassus, subaequalis, deorsum fulvo-fibrillosus; sporae 10–12.5× 5.5–7 μ; pleurocystidia 38–52 × 12–18 μ, ventricosa et capitata; cheilocystidia 40–65 × 9–15 μ, cylindrica et capitata. Specimen typicum in Herb. Univ. Mich. conservatum; legit Jackson Guard Station, Hoh River, Olympic National Park, Wash., May 13, 1939, Smith n. *13398*.

Pileus 1.5–3 cm broad, obtusely conic becoming broadly conic or nearly plane with an obtuse umbo, glabrous, "tawny" when moist, translucent striate, hygrophanous and fading to "ochraceous buff"; flesh concolorous with surface, thin but not fragile, odor not distinctive, taste *bitter;* lamellae close (± 20 reach the stipe) narrow to moderately broad (± 3 mm), squarely adnate but soon seceding, nearly equal but broadest near stipe, dark ochraceous tawny, pruinose under a lens, edges fimbriate; stipe 5–6 cm long, ± 2 mm thick, slightly enlarged at base, dark reddish brown over all (all mature), *surface covered to near apex with dingy tawny appressed fibrils,* apex pruinose.

Spores 10–12.5 × 5.5–7 μ, slightly inequilateral in side view, in face view ovate, dark tawny in KOH, warty in KOH but suprahilar depression smooth, apical pore present but minute; basidia two-spored, 26–29 × 7–8.5 μ, hyaline in KOH; pleurocystidia present, 38–52 × 12–18 μ, ventricose with broadly rounded apices or apices capitate and as broad as the ventricose part, thin-walled and hyaline in KOH; cheilocystidia abundant, 40–65 × 9–15 μ, ventricose to cylindric below a capitate apex, broadest at apex, hyaline as revived in KOH or with a dull ochraceous to brown content; gill trama interwoven, pale ochraceous tawny in KOH from incrusting pigment; pileus trama darker than gill trama, homogeneous, clamp connections present; pilocystidia scattered,

similar to cheilocystidia or with slightly thickened ochraceous walls at least near base; caulocystidia in fascicles,± like cheilocystidia but often more elongated, walls of some colored near base.

Habit, habitat and distribution: On conifer logs, Hoh River above Jackson Guard Station, Olympic National Park, Wash., May 13, 1939, Sm-*13398*.

Observations: This is a robust variety with tawny fibrils over lower half of stipe and with a tendency for many of the cheilocystidia to be colored. It may be a distinct species by virtue of the colored veil but I feel that more collections are needed to establish this.

19. **Galerina rudericola** sp. nov.

Pileus 10–25 mm latus, obtusus, margine primo involuto, demum pianoumbonatus, glaber; lamellae subdistantes vel distantes, subdecurrentes, angustae; stipes 3–5 cm longus, 1.5–2 mm crassus, aequalis, sursum fibrilloso-annulatus, deorsum demum fusco-brunneus; sporae 7–9 × 4–5 μ; pleurocystidia 40–50(–60) × 10–15(–18) μ, fusoidi-ventricosa. Specimen typicum in Herb. Univ. Mich. conservatum; legit prope Green Lake, Mt. Rainier National Park, Wash., Oct. 17, 1952, Smith n. *41070*.

Pileus 10–25 mm broad, obtuse with an incurved margin young, expanding to plane with a conic umbo or the margin remaining decurved, surface glabrous, moist, "buckthorn brown" fading to near "pinkish buff" or pale pinkish buff, faintly striate moist; odor and taste not recorded; lamellae subdistant to distant, arcuate to short-decurrent, narrow, dingy ochraceous tawny, edges white-fimbriate; stipe 3–5 cm long, 1.5–2 mm thick, equal, fragile, with a submembranous to fibrillose superior annulus, punctate-fibrillose and buff colored above the ring, appressed fibrillose and dull honey color below, darkening from the base up in age.

Spores 7–9 × 4–5 μ, in side view obscurely inequilateral, in face view elliptic to narrowly ovate, minutely roughened and with a ragged line around the smooth depression but *no* blisters, color dark ochraceous tawny in KOH; basidia four-spored; pleurocystidia scattered, 40–50(–60) × 10–15(–18) μ, fusoid-ventricose, hyaline or with yellowish walls (in KOH), with obtuse apices but occasionally apex with a nipple-like protrusion either apical or to one side, or apex subcapitate; cheilocystidia similar to pleurocystidia or necks more flexuous and elongated; gill trama subparallel, subhymenium not distinctive; pileus trama homogeneous (no pellicle seen); clamp connections present.

Habit, habitat and distribution: On lignicolous debris at base of an old avalanche now well grown over the herbaceous plants and shrubs, Green Lake, Mt. Rainier National Park, Wash., Oct. 17, 1952, Sm-*41070*.

Observations: The fruiting bodies look like those of a very slender form of *G. marginata* but the fibrillose annulus and minutely asperulate spores with their smooth plage readily distinguish it. The colors of the pileus are dingy ocher brown, quite different from the typical ochraceous tawny to tawny of the *G. hypnorum* series. It differs sharply from *G. subochracea* in lacking a gelatinous pellicle or even a lubricous feel, as well as in the darkening stipe.

20. **Galerina rugisperma** sp. nov.

Pileus 8–15 mm latus, subplanus, pallide fulvus; lamellae latae, anguste adnatae; stipes 2–4 cm longus, c. 1 mm crassus, aequalis, deorsum badius; sporae 8–10 × 5–6 μ; cheilocystidia 38–60 × 8–13 μ, anguste ventricosa et capitata vel subcylindrica et capitata. Specimen typicum in Herb. Univ. Mich. conservatum; legit ad terram, Longmire, Wash., Sept. 22, 1952, Smith n. *40146*.

Pileus 8–15 mm broad, obtuse, expanding to plane or nearly so, surface glabrous, moist, hygrophanous, striate, ochraceous tawny fading to pinkish buff but drying darker; lamellae broad, horizontal, narrowly adnate, edges slightly fimbriate, faces ochraceous tawny at maturity; stipe 2–4 cm long, ± 1 mm thick, equal, pale honey color above, becoming reddish brown from base up in age, veil thin and leaving scattered appressed patches of fibrils over lower portion.

Spores 8–10 × 5–6 μ, inequilateral in side view, in face view ovate, nearly russet in KOH, with a wrinkled outer layer variously loosened over the surface; basidia four-spored; pleurocystidia none; cheilocystidia abundant, 38–60 × 8–13 μ, ventricose to narrowly ventricose and with a capitellum 4–7 μ broad, or subcylindric-capitate, hyaline but as revived in KOH with a coloidal content; gill trama somewhat interwoven, ochraceous in KOH; pileus trama homogeneous, ochraceous in KOH; clamp connections present.

Habit, habitat and distribution: Gregarious on wet earth along edge of swamp, Longmire, Wash., Sept. 22, 1952, Sm-*40146*.

Observations: The large, capitate cheilocystidia, very loose episporium of the spore, darkening stipe, obtuse pileus and thin

veil are characteristic. In spore characters it is distinct from the *G. hyponorum* complex.

21. **Galerina subdecurrens** sp. nov.

Pileus 5–15 mm latus, late convexus, argillaceus, glaber; lamellae distantes vel subdistantes, subdecurrentes, latae; stipes 10–25 mm longus, 1 mm crassus, deorsum brunnescens et cinereo-fibrillosus; sporae 9–12.5 × 4–4.8 μ, leves; cheilocystidia 20–30 × 6–9 μ anguste ventricosa. Specimen typicum in Herb. Univ. Mich. conservatum; legit ad terram, Lower Tahoma Creek, Mt. Rainier National Park, Wash., Oct. 20, 1952, Smith n. *41225*.

Pileus 5–15 mm broad, obtuse to convex with young, expanding to broadly convex, surface glabrous, moist, hygrophanous, dingy ochraceous tawny to clay color moist, dingy pinkish buff faded, striate when moist, flesh thin and fragile, odor and taste not recorded; lamellae broad, subdistant to distant, broadly adnate to subdecurrent (approaching the condition found in *Xeromphalina picta*), seceding at times, edges even, dingy buff at first, ± ochraceous tawny when mature; stipe short, 10–25 × ± 1 mm, equal, pale honey color above, dark reddish brown below and lower half with pallid to grayish appressed fibrils (veil remnants?).

Spores (9–)10–12.5(–13) × 4–4.8 μ, narrowly oblong, some in face view narrowly ovate, *smooth*, pale cinnamon in KOH, and becoming pale ochraceous on standing, apical pore present but apex not truncate under ordinary magnifications, obscurely so under oil immersion; basidia four-spored, hyaline in KOH; pleurocystidia none; cheilocystidia fusoid ventricose, *small*, 20–30 × 6–9 μ, narrowly ventricose and apices obtuse; gill trama hyaline in KOH, somewhat interwoven; pileus trama homogeneous in structure but hyphae of upper half with ochraceous incrusting pigment; clamp connections present.

Habit, habitat and distribution: Cespitose-gregarious under alder on soil at edge of road, Lower Tahoma Creek (old camp ground), Mt. Rainier National Park, Oct. 20, 1952, Sm-*41225*.

Observations: The long, narrow, smooth, pale cinnamon to ochraceous spores, thin veil, and darkening stipe are distinctive. When viewed under oil a very faint ragged line can be made out delimiting the suprahilar area, but it cannot be demonstrated on all spores. I have demonstrated it under a 90 ×, 1.30 NA oil immersion objective. The species is thus a typical *Galerina* as interpreted here even though the apical pore is broader than usual

and reminds one of that found in *G. stagnina*. This type of apical pore occurring in a species in which the "plage" is still faintly delimited indicates the relationship of the *G. stagnina* stirps to the typical species of *Galerina*.

22. **Galerina subfiliformis** sp. nov.

Pileus 5–8 mm latus, 6–8 mm altus, acute conicus, dilute argillaceus, glaber; lamellae subdistantes, adnatae, latae; stipes 1–3 cm longus, subfiliformis, dilute ochraceus, sparsim fibrillosus; sporae 10–14 × 6–7 μ, *leves;* cheilocystidia 28–37 × 6–9 μ, fusoidi-ventricosa. Specimen typicum in ligni Tsagae, St. Andrew's Creek, Mt. Rainier National Park, Wash., Oct. 5, 1952, Smith n. *40607*.

Pileus 5–8 mm broad and 6–8 mm high, sharply conic, remaining unexpanded, surface glabrous, moist, hygrophanous, striate when moist, dingy yellowish to near cinnamon-buff moist, pale pinkish buff to whitish faded; odor and taste not recorded; lamellae broad, ascending adnate, subdistant to distant, pale ochraceous tawny in age; stipe 1–3 cm long, almost filiform (less than 1 mm thick) equal, fragile, very pale honey color over all, apex pruinose, base with pallid fibrils from the thin veil.

Spores 10–14 × 6–7 μ, pale ochraceous tawny in KOH, smooth, ovate in face view, obscurely inequilateral in side view; basidia four-spored; pleurocystidia none; cheilocystidia 28–37 × 6–9 μ, fusoid-ventricose with narrow necks and unenlarged apices; gill trama somewhat interwoven, hyaline in KOH; pileus trama homogeneous, hyaline to yellowish in KOH; clamp connections present.

Habit, habitat and distribution: Scattered on mossy conifer logs, St. Andrew's Creek, Mt. Rainier National Park, Wash., Oct. 5, 1952, Sm-*40607*.

Observations: The large, pale ochraceous tawny, smooth spores, pale pileus and stipe, and thin veil are distinctive along with the small conic cap. It fruits on wet logs in cold localities. It has the aspect of *G. hypnorum* but the smooth spores distinguish it.

23. **Galerina subochracea** sp. nov.

Pileus 6–15 mm latus, obtuse conicus demum late convexus, glaber, *subviscidus,* pallide fulvus; stipes 3–5 cm longus, 1–2 mm crassus, fragilis, luteus, fibrilloso-annulatus; sporae 8–9.5 × 4.5–5.2 μ; pleurocystidia 45–66 × 10–18 μ, fusoidi-ventricosa. Specimen typicum in Herb. Univ. of Mich. conservatum; legit prope Salmon River, Mt. Hood, Ore., Oct. 10, 1946, Smith n. *24347*.

Pileus 6–15 mm broad, obtusely conic to convex, expanding to plane, the margin connivent at first, surface lubricous to *viscid to the touch*, glabrous or with veil remnants scattered along the margin at first, hygrophanous, "ochraceous tawny" moist, fading to orange buff or paler; flesh thin and fragile, odor and taste not recorded; lamellae moderately broad, broadly adnate, close to subdistant, pale ochraceous tawny with white fimbriate edges; stipe 3–5 cm long, 1–2 mm thick, equal or nearly so, tubular, fragile, yellow over all at first and with a *distinct medium fibrillose annulus*, with scattered fibrils or patches below, somewhat darkening from base upward in age, but tending to fade again in drying.

Spores 8–9.5 × 4.5–5.2 μ, tawny ochraceous in KOH, inequilateral in side view, ovate in face view, exospore very minutely roughened, smooth suprahilar depression evident, pore apical and distinct; basidia 23–26 × 7–8 μ, four-spored, sterigmata very indistinct; *pleurocystidia abundant*, 45–66 × 10–18 μ, fusoid-ventricose with obtuse to subacute apices; cheilocystidia abundant, similar to pleurocystidia or slightly more variable in shape; gill trama parallel to somewhat interwoven, pale yellow to hyaline in KOH; pileus trama with a thin surface layer of hyphae 5–7 μ diam. with incrusted walls, these hyphae quite contorted and somewhat gelatinous, remainder of flesh homogeneous, yellow in KOH near pellicle, nearly hyaline toward subhymenium; clamp connections present; no pilocystidia seen.

Habit, habitat and distribution: Scattered on debris at edge of beaver pond, East Fork of Salmon River, Mt. Hood, Oregon, October 10, 1946, coll. Wm. B. Gruber (Sm-*21347*). An additional collection referred here is Sm-*19904*, from debris near Rhododendron, Oregon, Oct. 18, 1944.

Observations: The diagnostic characters are the viscid cap, fibrillose annulus, small nearly smooth spores with a smooth suprahilar depression and conspicuous pleurocystidia. The convex to plane pileus is an additional field character. In the dried state the fruiting bodies are concolorous throughout. The darkening of the stipe, as noted on the fresh material, fades out in drying. Although related to *G. marginata* in spore and cystidial characters, one would be more inclined to class *G. subochracea* as a *Cortinarius* in the field. The smooth suprahilar depression of the spore, of course, makes a reference to *Cortinarius* untenable. Actually there are a number of characters which separate it from *G. marginata* and forms. The annulus is a fibrillose zone, the

pleurocystidia are shorter and more ventricose, the spores and tramal tissues paler in KOH, and the darkening of the stipe is not so pronounced and is not retained in drying. In one collection (Sm-*19904*) the pileus was merely lubricous, but in view of the situation in *G. unicolor,* as regards the pellicle, no emphasis is given the difference in viscidity in the two collections described as *G. subochracea.* The veil in collection *19904* dried yellowish, but notes on the color of the veil when fresh were not taken for either collection.

24. **Galerina tahquamenonensis** sp. nov.

Pileus 8–15 mm latus, campanulatus, subbadius, margine appendiculatus; lamellae subdistantes, latae, adnatae; stipes 1–2 cm longus, 1–1.5 mm crassus, subaequalis, deorsum subbadius, peronato-fibrillosus, sursum fibrilloso-annulatus; sporae 7–9(–10–12) × 5–6.5(–7) μ (basidia 1-, 2-, 3-, 4-spora); cheilocystidia 24–36 × 9–11 μ, ventricose et acuta vel subcapitata. Specimen typicum in Herb. Mich. conservatum; legit in ligno *Fagi,* Tahquamenon Falls State Park, Mich., July 2, 1952, Smith n. *39045.*

Pileus 8–12(–15) mm broad, obtusely conic with a ± incurved margin, expanding to campanulate or remaining convex, glabrous except for margin which is at first appendiculate with patches of the thin veil (± dentate-appendiculate), veil remnants soon evanescent, color when young and moist "Mars brown," soon "cinnamon brown" but the margin becoming dingy ochraceous tawny and striate, hygrophanous, fading to dingy cinnamon buff or paler; flesh concolorous with pileus, soft, taste and odor not distinctive; lamellae broadly adnate, broad, nearly subdistant at first, truly subdistant at maturity, ochraceous tawny (paler than pileus), edges even; stipe short, ± 1–2 cm long, ± 1–1.5 mm thick, equal or enlarged below, concolorous with pileus below, apex soon fading out to almost concolorous with the gills but in age again often darker, *peronate-fibrillose* up to the superior *thin often fragmentary annulus,* pruinose above, in age glabrescent.

Spores 7–9(–10–12) × 5–6.5(–7) μ, ovoid to subelliptic, *roughened at least around the suprahilar plate,* ochraceous tawny when revived in KOH; basidia 1–2–3–4-spored; *pleurocystidia* none seen; cheilocystidia 24–36 × 9–11 μ, ventricose with a narrow neck and sometimes with a small capitellum, wall of capitellum thickened in some and highly refractive; gill trama parallel, the cells short-ellipsoid, tawny to rusty brown in KOH, subhymenium not distinctive; pileus trama dark rusty brown in KOH and homogeneous, pigment incrusted on walls of hyphae; clamp connections present.

Habit, habitat and distribution: Gregarious on rotten hardwood, Tahquamenon Falls State Park, Michigan, July 2, 1952, Sm-*39045*. Sm-*37025*, July 23, 1951 and Sm-*38355*, Aug. 28, 1951 were also from the Park and on the same substratum.

Observations: No pilocystidia were observed. The stature of this species is that of *G. triscopoda* but the well-developed veil readily distinguishes it. The roughness of the spores is best seen under oil immersion. The smooth suprahilar depression is indistinctly bounded in most spores but can be seen in enough to verify its presence. The species appears to be most closely related to *G. camerina* sensu Kuhner (l.c., p. 213) in the characters of spores and cheilocystidia, but differs in having a peronate veil and usually in being annulate, and in growing on hardwood logs.

25. **Galerina umbrinipes** sp. nov.

Pileus 6–10 mm latus, conicus, demum campanulatus, glaber, pallide fulvus, striatus; lamellae adnatae, subdistantes, angustae demum latae; stipes 3–4 cm longus, 1–1.5 mm crassus, aequalis, *umbrinus;* sporae 10–12.5(–13) × 5.5–7 μ; pleurocystidia diversa: 1) 23–30 × 5–6 μ, fusoidi-ventricosa, 2) 46–80 × 9–14 μ, ventricosa et subcapitata. Specimen typicum in Herb. Mich conservatum; legit prope Still Creek, Mt. Hood National Forest, Oregon, Oct. 3, 1946, Smith n. *24051*.

Pileus 6–10 mm broad, obtusely conic, remaining conic or becoming campanulate, glabrous, moist, hygrophanous, "ochraceous tawny" moist, pale buff faded, when moist translucent-striate to the waters disc, somewhat sulcate faded; flesh very thin and fragile, no appreciable odor; lamellae ascending-adnate, narrow to moderately broad, 1 tier of lamellulae, subdistant, ochraceous tawny; stipe 3–4 cm long, 1–1.5 mm thick, equal or base slightly enlarged, *pale umber over all when young*, in age fading out to more or less concolorous with pileus in upper part, umber cast remaining at base, surface finely pubescent over all from projecting caulocystidia.

Spores 10–12.5(–13) × 5.5–7 μ, tawny to pale tawny in KOH, inequilateral in side view, ovate in face view, outer wall wrinkled, suprahilar area slightly roughened and not noticeably depressed, apical pore very minute, not readily visible except under oil; basidia four-spored, 23–26 × 6–7 μ, hyaline in KOH, not projecting when sporulating; pleurocystidia of two types, the first rare (possibly immature stages of second type) 23–30 × 5–6 μ, fusoid and imbedded in hymenium, hyaline and homogeneous in KOH, the second abundant, greatly elongated 46–80 × 9–14 μ, ventri-

cose at base, apex subcapitate to subacute, hyaline, thin-walled in KOH or walls slightly thickened; cheilocystidia similar to pleurocystidia, very abundant; gill trama subparallel, the cells short and broad, with ochraceous encrusting pigment on the walls as revived in KOH, yellow in water mounts of fresh material; pileus trama homogeneous, pellicle undifferentiated, cells more compactly interwoven near surface than they are deeper down, the walls yellow in KOH from encrusting pigment; caulocystidia fusoid ventricose, hyaline, thin-walled in KOH, (40–)50–100 × 12–18 μ, ventricose at base, tapered to a subcapitate to subacute apex.

Habit, habitat and distribution: Gregarious on moss under conifers, Still Creek Forest Camp, 3800 ft. elev., Mt. Hood National Forest, Washington, Oct. 3, 1946, Sm-*24051*.

Observations: The umber stipe is an unusual character in this genus, particularly on young specimens. It is likely that very small buttons have a similar cast to the pileus. The young stipe may appear almost fibrillose at times but an examination under the microscope shows the fibrils to be elongated caulocystidia. The spores are essentially as in *G. rubiginosa,* but the color of the stipe when young and the presence of small pleurocystidia along with the large ones are a pair of characters which prevent *G. umbrinipes* from being attached to *G. rubiginosa* as a taxon below the rank of species. The small pleurocystidia are indeed curious; when forked they resemble 2-spored basidia with thick, obtuse sterigmata.

26. **Galerina vaccinii** sp. nov.

Pileus 6–12(–15) mm latus, campanulatus, glaber, udus et striatus, sicco fibrillosus, subfulvus; lamellae confertae demum distantes, late adnatae, pallide argillaceae; stipes 10–20 mm longus, c. 1 mm crassus, aequalis, fragilis, deorsum brunnescens, sparsim fibrillosus; sporae 10–12.5 × 6–7 μ; cheilocystidia 38–50 × 9–12 μ, fusoidi-ventricosa vel subcylindrica. Specimen typicum in Herb. Univ. Mich. conservatum; legit sub *Vaccinio delicioso* prope Snow Lake, Mt. Rainier National Park, Wash., Oct. 11, 1952, Helen V. Smith (A. H. Smith n. *40798*).

Pileus 6–12(–15) mm broad, obtuse when young, becoming obtusely campanulate to plano-umbonate or umbo obsolete, dull tawny to dark ochraceous tawny and widely striate moist, near cinnamon buff faded, appearing glabrous when moist but when faded appearing fibrillose under a lens; flesh thin and very fragile, odor and taste not distinctive; lamellae close or finally distant,

broadly adnate or with a decurrent tooth, broad, *cinnamon-buff* when mature, paler when young, edges faintly fimbriate; stipe 10–20 mm long, ± 1 mm thick, equal, fragile, concolor with pileus but base darkening in age, thinly fibrillose over lower part from the pallid remnants of a veil, glabrescent.

Spores 10–12.5 × 6–7 μ elongate ovate in face view, obscurely elongate-inequilateral in side view, *smooth* but with a faint ragged line marking the boundary of the suprahilar depression, ochraceous tawny or slightly darker in KOH, in water mounts of fresh material pale ochraceous; basidia 4-spored; pleurocystidia none, a few ocher-brown basidioles present in hymenium; cheilocystidia 38–50 × 9–12 μ, fusoid-ventricose to subcylindric with obtuse apices, abundant; gill trama somewhat interwoven, ochraceous in KOH, the hyphal cells enlarged; pileus trama homogeneous but cells near surface enlarged giving almost the appearance of sphaerocysts, pilocystidia present as hyphal end-cells, trama rusty in KOH from heavy pigment incrustations; clamp connections present.

Habit, habitat and distribution: Cespitose-gregarious on duff under *Vaccinium deliciosum*, Snow Lake, Mt. Rainier National Park, Wash., Oct. 11, 1952, coll. Helen V. Smith (AHS-*40798*).

Observations: The spores are more like those of *Galerina* than *Cortinarius*, and since cheilocystidia are typical of the former, the collection is placed there. The dried specimens, however, remind one of the latter genus.

27. **Galerina venenata** sp. nov.

Pileus 1–3.5 latus, late convexus demum planus vel depressus, glaber, udus, cinnamomeus, hygrophanus, deinde pallidus; sapor farinaceus, demum amarus; lamellae latae, subdistantes, late adnatae, fulvae; stipes 3–4 cm longus, 3–5 mm crassus, deorsum sub-clavatus; anulatus; sporae 8–11 × 6–6.5 μ; pleurocystidia 45–70 × 9–15 μ; cheilocystidia pleurocystidiis similia. Specimen typimum in Herb. Univ. Mich. conservatum; lectum Portland, Ore., Jan., 1953.

Pileus 1–3.5 cm broad, broadly convex young, expanding to plane or with a slightly uplifted, arched margin and depressed disc, surface glabrous and moist, near cinnamon brown, hygrophanous, fading to a very dingy yellowish white to pinkish buff (often browner because of deposited spores), margin crenate to lacerated; flesh moderately thick, odor farinaceous, taste farinaceous but slowly becoming bitter and disagreeable, leaving an extended dryness in the throat; lamellae *broad*, subdistant, *broadly* adnate, golden tawny becoming dull cinnamon, edges even; stipe 3–4 cm

long, 3–5 mm at apex, enlarged somewhat toward the base, brownish, with a very thin, apical annulus appressed against the stipe in about half the specimens, glabrous except for the white-mycelioid base.

Spores 8–11× 6–6.5 μ, subinequilateral in side view, ovate in face view, russet in KOH, with a conspicuously rugulose-warty outer wall tending to loosen to form blisters near hilar end, suprahilar depression smooth; basidia four-spored, content oily-globular, 20–25 × 7–8 μ; pleurocystidia present, 45–70 × 9–15 μ, hyaline in KOH, smooth, fusoid-ventricose with acute to subacute apices; cheilocystidia similar to pleurocystidia, no clavate to mucronate cells seen; gill trama somewhat interwoven, ochraceous in KOH, hyphal cells up to 30 μ broad, hymenopodium not distinctive; pileus trama homogeneous, the hyphae at the surface 6–12(–15)μ in diam. and interwoven, tramal body ochraceous in KOH; clamp connections present.

Habit, habitat and distribution: Gregarious on a lawn, Portland, Oregon, Jan. 24–27, 1953. Coll. by members of the Oregon Mycological Society.

Observations: This fungus was positively identified by a man and his wife as the fungus they ate a short time before being stricken with a severe case of fungous poisoning. The clinical history of this case is being published by Dr. Grossman of Portland, Ore.

There has been discussion of the edible and poisonous properties of the *Galerina marginata* complex in the literature (Harding, doctoral thesis, 1952). *"Pholiota autumnalis"* was blamed for a case in Minnesota, and Harding showed clearly that in this case a number of species of fungi (at least 2) had served to confuse the issue. As a result of his study he was inclined to blame a species of *Gymnopilus* as the poison species and cited work he had done on *Galerina marginata* which supported the conclusions of European authors that it is an edible fungus. He regarded *G. autumnalis* as a synonym of *G. marginata.*

The discovery of this poisonous fungus in Portland, Ore. reopens the whole matter for additional consideration. First, the possibility still exists that *Galerina autumnalis,* as recognized by us, may be a poisonous species. We do not dispute the claim that *G. marginata* is edible. That seems to be well established. How-

ever, from the data at hand we are forced to the conclusion that a second poisonous species in the group does exist, namely *G. venenata*. Since the latter has a very different cap cuticle from *G. autumnalis* we cannot consider it as a variety or form of Peck's fungus. In addition there is the difference in the shape of the apex of the cystidia and in the habitat. The very thin veil of *G. venenata* leaves only a most inconspicuous zone, and finally, the dried fruiting bodies do not compare at all favorably.

Because of the situation in regard to edibility in this group, we believe it is desirable to search for all characters which might be of use taxonomically in the hope of eventually being able to distinguish the poisonous from the edible members on correlated characters even though these may seem trivial in themselves. For this reason Smith and Singer have re-examined all the species placed in synonymy with *G. marginata* by Harding, and are recognizing them if there seems to be the slightest justification for doing so. We have examined the collection of the *Galerina* sent to Kauffman by Dr. Whetstone, and, though it is poorly dried, remains of a gelatinous pellicle were seen. Hence we classify this collection in *G. autumnalis*.

28. **Galerina vialis** sp. nov.

Pileus 1–4 cm latus, cinnamomeo-brunneus, glaber, hygrophanus, valde striatus; lamellae subdecurrentes, confertae vel subdistantes, subfulvae; stipes 3–5 cm longus, 2–4 mm crassus, deorsum valde brunnescens et peronato-fibrillosus, sursum annulatus; sporae 8.5–11 ×5–6.5 μ; pleurocystidia in KOH subochracea. Specimen typicum in Herb. Univ. Mich. conservatum; legit ad viam prope Carbon River, Mt. Rainier National Park, Wash., Oct. 8, 1952, A. H. Smith n. *40709*.

Pileus 1–4 cm broad, convex when young, becoming plane or the disc shallowly depressed in age and margin arched, surface moist and hygrophanous, color evenly "cinnamon-brown" moist, gradually dingy ochraceous tawny before fading, fading on disc first to ochraceous buff but finally about pale pinkish buff translucent-striate nearly to disc when moist, glabrous but in age when faded ± squamulose from breaking of cuticle; flesh thin, soft, fragile, odor and taste not distinctive; lamellae broadly adnate to slightly decurrent, seceding, close to subdistant, broad, dull ochraceous tawny, edges minutely fimbriate; stipe 3–5 cm long, 2–4 mm at apex, narrowly clavate to nearly equal, concolorous with young gills above but base soon bister and discoloration progressing up-

ward, annulus superior, gray-fibrillose below annulus or fibrils breaking into squamules, coarsely pruinose-fibrillose above.

Spores 8.5–11 × 5–6.5 μ, in face view ovate and many with an almost snout-like apex, inequilateral in side view, rugulose-roughened and with "blisters" around the smooth suprahilar depression, russet in KOH; basidia 26–30 × 7.5–8 μ; pleurocystidia scattered 48–60 × 12–18 μ, fusoid-ventricose with subacute apices and the walls of the neck flexuous, thin-walled or walls slightly thickened, *dingy yellowish* to nearly hyaline in KOH, smooth; cheilocystidia abundant and fully as large or larger than the pleurocystidia, ventricose portion pale dingy yellow in KOH; gill trama subparallel, the cells ± ellipsoid, nearly hyaline in water when fresh, ochraceous to cinnamon-buff as revived in KOH; pileus trama homogeneous, the cuticular layer of nongelatinous hyphae, when revived in KOH the surface layer yellowish, a cinnamon colored layer beneath this and the remainder nearly hyaline; clamp connections present.

Habit, habitat and distribution: Gregarious-subcespitose along a road on soil, Carbon River, Mt. Rainier National Park, elev. 3300 ft., Oct. 8–9, 1952, Sm-*40709, 40779*.

Observations: This species is in the *G. marginata* complex but is readily distinct when fresh by the dark brown pileus, more fragile consistency, and more conspicuous striae. Under the microscope the pale smoky-yellow cystidia are distinctive of revived material. Their walls are slightly thickened in the midportion and the yellow pigment is in the wall. As compared with *G. marginata* the fibrillose sheath over the lower portion of the stipe is much looser in organization, thicker, and in age leaves the lower part of the stipe with squamules or zones before becoming glabrous. Not much emphasis should be placed on the habitat being terrestrial because it is entirely possible that considerable wood has been used as a fill in making the road.

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MYXOMYCETES OF LOUISIANA

BERNARD LOWY

(WITH 10 FIGURES)

The mycological flora of Louisiana, particularly the saprobic forms, has not been extensively explored and it therefore seems desirable that some contribution be made toward a systematic treatment of the fungi of this region.

The earliest published report on Louisiana fungi was made by A. Featherman (1) in 1871 in the form of a brief list of various species, mostly agarics, collected in Baton Rouge. A single Myxomycete, *Fuligo septica* (as *Aethalium septicum*), was named. Langlois (2), in 1878, published a more extensive record of the cryptogamic flora of the state, in which ten Myxomycetes were identified, but among the many subsequent publications on the fungi of this area no further account of them has appeared.

The present paper includes 47 species of Myxomycetes, of which number 42 are reported from Louisiana for the first time. Twenty genera are represented. Almost all the collections were made within a radius of about one mile of the University campus, chiefly between the months of February and August of 1952. As the area of search is extended to other parts of the state, it is to be anticipated that additional species will be encountered.

Collections were found growing on dead wood, unless otherwise indicated. All specimens are deposited in the mycological herbarium of Louisiana State University.

CERATIOMYXALES

Ceratiomyxaceae

Ceratiomyxa fruticulosa (Mull.) Macbr.

Frutifications extensive, branching 3–5 mm tall, covering about one-half meter of a fallen log; spores hyaline, ellipsoid, 10–12 × 6 μ

in diameter. This collection corresponds rather closely with the description given by Lister (3) of the "variety" *flexulosa*. The species is quite variable in morphology and pigmentation, but, as Martin (4) has noted, "the varieties seem unworthy of recognition."

LICEALES

Liceaceae

Tubifera ferruginosa (Batsch) J. F. Gmel.

RETICULARIACEAE

Enteridium Rozeanum (Rost.) Wingate
Lycogala Epidendrum (L.) Fries FIG. 2.
Lycogala exiguum Morgan
Lycogala flavofuscum (Ehrenb.) Rost. FIG. 1.
Reticularia Lycoperdon Bull.

CRIBRARIACEAE

Dictydium cancellatum (Batsch) Macbr.
Lindbladia effusa (Ehrenb.) Rost. FIG. 3.

TRICHIALES

Trichiaceae

Arcyria cinerea (Bull.) Pers. FIG. 9.
Arcyria denudata (L.) Wettst.
Arcyria incarnata (Pers.) Pers.
Arcyria insignis Kalchbr. & Cooke
Arcyria nutans (Bull.) Grev.
Hemitrichia Serpula (Scop.) Rost.
Hemitrichia stipitata (Schw.) Macbr.
Hemitrichia Vesparium (Batsch) Macbr. FIG. 4.
Oligonema flavidum (Peck) Peck FIG. 5.
Oligonema Schweinitzii (Berk.) G. W. Martin
Perichaena vermicularis (Schw.) Rost.
Trichia erecta Rex
Trichia varia (Pers.) Pers.

FIG. 1. *Lycogala flavofuscum*, × 2. FIG. 2. *Lycogala Epidendrum*, × 3.
FIG. 3. *Lindbladia effusa*, × 1.

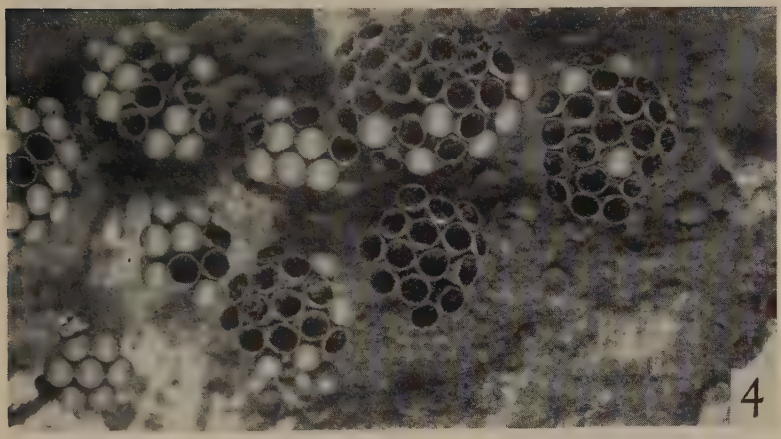

FIG. 4. *Hemitrichia Vesparium*, × 10. FIG. 5. *Oligonema flavidum*, × 20.

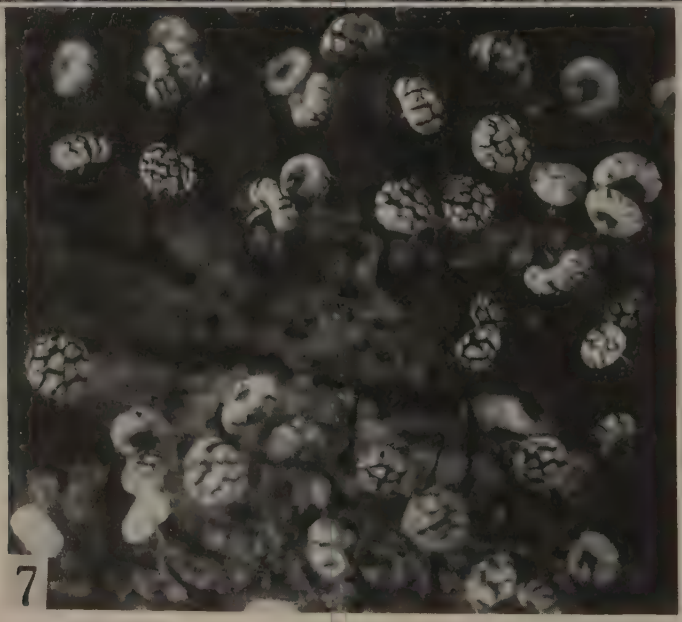

FIG. 6. *Physarum Serpula*, × 5. FIG. 7. *Physarum viride*, × 15.

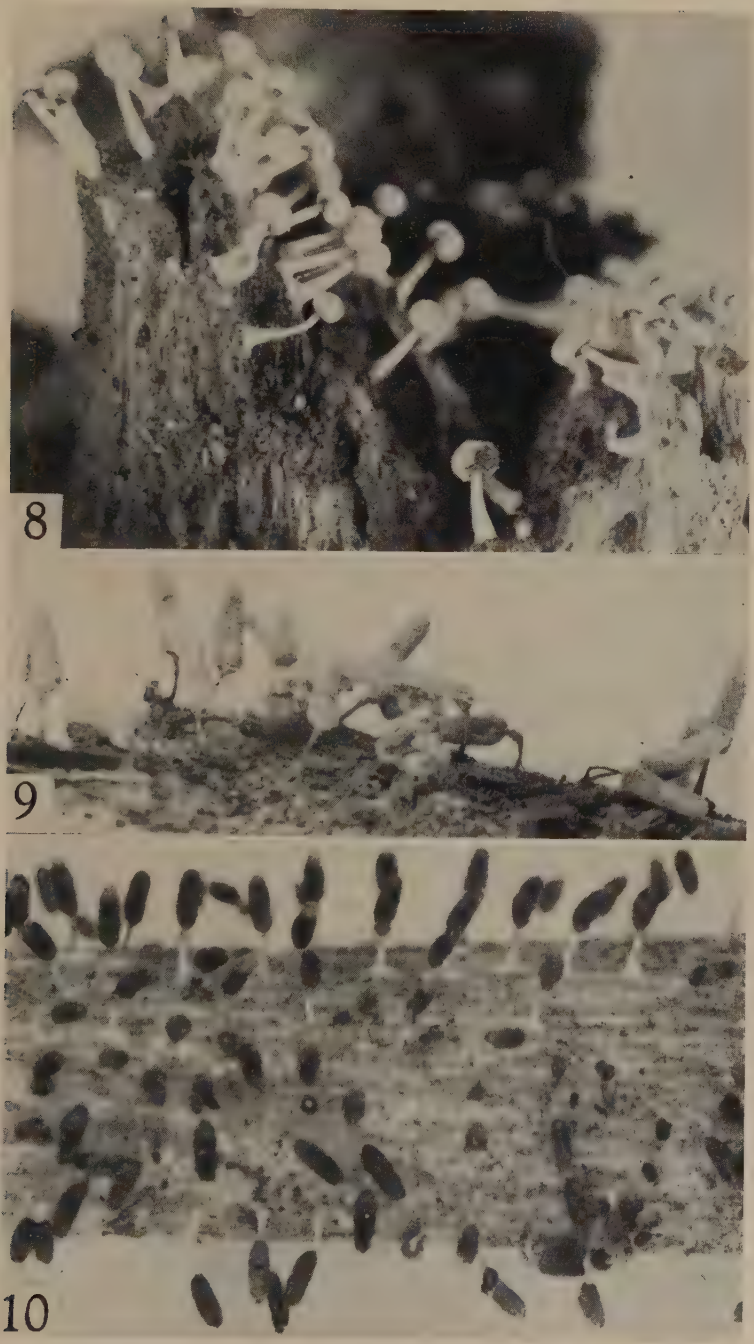

FIG. 8. *Physarum globuliferum*, × 10. FIG. 9. *Arcyria cinerea*, × 10. FIG. 10. *Diachea leucopodia*, × 15.

STEMONITALES

Stemonitaceae

Comatricha longa Peck
Comatricha typhoides (Bull.) Rost.
Diachea leucopodia (Bull.) Rost. FIG. 10.
Stemonitis axifera (Bull.) Macbr.
Stemonitis carolinensis Macbr.
Stemonitis confluens Cooke & Ellis
Stemonitis fusca Roth
Stemonitis hyperopta Meylan
Stemonitis nigrescens Rex
Stemonitis splendens Rost.
Stemonitis virginiensis Rex
Stemonitis Webberi Rex

PHYSARALES

Physaraceae

Badhamia gracilis (Macbr.) Macbr.
Craterium leucocephalum (Pers.) Pers.
Fuligo septica (L.) Weber
Physarella oblonga (Berk. & Curtis)Morgan
Physarum bogoriense Racib.
Physarum cinereum (Batsch) Pers.
Physarum Didermoides (Pers.) Rost.
Physarum globuliferum (Bull.) Pers. FIG. 8.
Physarum nutans Pers.
Physarum polycephalum Schw.
Physarum pusillum (Berk. & Curtis) G. Lister
Physarum Serpula Morgan FIG. 6.
Physarum viride (Bull.) Pers. FIG. 7.

Supplementary note: After the manuscript of this paper had gone to the printer, further collections were made which included nine species not previously reported. It is thought best to include these at this time, increasing the total number of species listed to 56 and the number of genera to 21.

TRICHIALES

Trichiaceae

Arcyria Oerstedtii Rost.
Arcyria occidentalis (Macbr.) G. Lister
Arcyria versicolor Phill.
Hemitrichia abietina (Wigand) G. Lister
Hemitrichia leiocarpa (Cooke) Lister

STEMONITALES

Stemonitaceae

Comatricha lurida Lister

PHYSARALES

Physaraceae

Physarum Bethelii Macbr.
Physarum mutabile (Rost.) G. Lister

Didymiaceae

Didymium Iridis (Ditmar) Fries

The writer wishes to acknowledge the help of Dr. G. W. Martin, who verified the identifications of several of the specimens in this report.

All photographs except that of *Lindbladia effusa* were taken by the author. Thanks are due Dr. E. C. Tims for this print.

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BOTRYOTRICHUM AND COCCOSPORA

MARY H. DOWNING

(WITH 1 FIGURE)

In attempting to identify three of the organisms in the Quartermaster Collection, White and Downing (**1951**) decided that they were conspecific with *Coccospora agricola* Goddard. In the absence of type material, the decision was based on a comparison of these cultures with the description and figures given by Goddard. It was felt that the species *C. agricola* was re-established, although the validity of *Coccospora* Wallr. was questioned at the time. Further investigation reveals that *Coccospora agricola* is a synonym of *Botryotrichum piluliferum* Sacc. and March. and new morphological observations make an emendation of *Botryotrichum* desirable.

The essential characteristics of *Botryotrichum piluliferum* as understood to this time are:

(1) *Spores* hyaline, globose, thick-walled, 10–25 μ in diameter, borne singly or in small clusters.

(2) *Hairs* prominent, rough, olivaceous, borne singly or in tufts.

Coccospora agricola Goddard, as described and illustrated, contains no reference to the sterile hairs. Of the three isolates (QM *336*, *337*, and *991*) described by White and Downing, two possess the sterile hair-like structures. Examination of an authentic herbarium specimen of Marchal's *Botryotrichum piluliferum* (FIG. 1, B, C), grown on agar, reveals prominent, rough, olivaceous hairs. Isolate QM *336* (FIG. 1, D, E, F, G) compares favorably in all respects with this specimen. Blochwitz (**1914**), in a study of *Botryotrichum piluliferum*, gave particular emphasis to the sterile hairs, and demonstrated their origin from the same hyphal base as that from which the spore structures arose. His isolate, from flypaper, has been preserved at the Centraalbureau voor

Schimmelcultures, and it still produces the typical aleuriospores and sterile hairs. This culture is also similar in appearance to QM *336*.

Something which seems to have been overlooked in all of the work reported above is the presence of another spore type. Study of the three cultures in our Quartermaster Collection reveals the presence of hyaline phialides, with phialospores produced in a single chain, which sometimes slimes down into a spherical mass. The phialospores are elliptic and hyaline, with thin smooth walls. The phialides are usually unbranched, short, and borne on the same aerial hyphae which produce the aleuriospores. The structure is most readily detected by direct examination of the petri dish culture under the low power of the compound microscope. Photomicrographs illustrate the two spore types arising from the same hyphae (FIG. 1, D, E. F). First noticed in a plate culture on leaves of *Phragmites communis* on mineral salts agar, the presence of phialides was later confirmed on potato dextrose agar. Within one week after inoculation, phialides begin to develop on the the aerial hyphae, producing rather long chains of spores. The phialospore form was seen in all three isolates, QM *336, 337, 991*, and in the Blochwitz culture.

White and Downing (**1951**) believed that the three Quartermaster isolates which they placed in *Coccospora agricola* Goddard have "close natural affinities with the two species upon which Traaen established his genus *Humicola*." Traaen (**1914**) described phialides in *Humicola*. The present discovery of phialides in *Botryotrichum piluliferum* is added support for the close relationship of *Humicola* and *Botryotrichum*. Isolates of both genera have been found to be cellulolytic; both are soil organisms with a preference for alkaline media (Jensen, **1931**; White and Downing, **1951**). The basis for separation now appears to be that *Botryotrichum piluliferum* is for the most part a hyaline counterpart of *Humicola*, having (usually) characteristic sterile hairs.

Since the present consideration reduces *Coccospora agricola* to the synonymy of *Botryotrichum piluliferum*, it seems that a redescription of *Botryotrichum* to include the phialospore form

FIG. 1, A–G. *Botryotrichum piluliferum* Sacc. and March. A. Original illustration reduced one-half from Bull. Soc. Roy. Bot. Belg. **24**: 66. B, C. Authentic specimen, Rabenhorst No. *3992*. B. Aleuriospores mounted in 7 percent KOH and stained with phloxine, × 500. C. Sterile hairs and aleuriospores, in Hoyer's mount stained with acid fuchsin, × 100. D, E, F, G. Culture QM *336* grown on a mineral salts agar containing a fragment of corn stalk and leaves of *Phragmites communis*, after 10 days incubation under room conditions; photographed directly from the petri dish surface under a compound microscope; D, E, F, × 550, illustrate the dual spore-bearing hyphae, with aleuriospores and phialospores produced on the same hypha; G, × 130, shows sterile hairs in a tuft, and aleuriospore racemes.

FIG. 1, H. *Botryotrichum* (*Peziotrichum*) *lachnella*, Saccardo's original illustration in Hedwigia (**32**: 56–59) reduced one-half.

should be in order. A modern concept of the species emending the original description follows.

BOTRYOTRICHUM Sacc. and March., Bull. Soc. Roy. Bot. Belg. **24**: 66. 1885. ill. *emend.*

B. PILULIFERUM Sacc. and March., Bull. Soc. Roy. Bot. Belg. **24**: 66. 1885. ill. *emend.*

Coccospora agricola Goddard, Bot. Gaz. **56**: 264–265. 1913. ill.

Fertile hyphae septate, hyaline, very little branched, usually aerial in character, 4–6 μ in diameter, bearing two types of spores. *Aleuriospores* acrogenous, borne either singly on short, undifferentiated side branches of septate hyphae, 12–30 μ long, or in whorls or in racemose clusters of spores in grape-like "bunches"; aleuriospores hyaline, globose, 10–21 μ in diameter, smooth, with walls up to 3.5 μ thick, surrounded by a hyaline membrane which commonly separates from the main wall and ruptures in mounts; Tilleul-buff (Ridgway) in color (in mass). *Phialides* borne at varying intervals, sometimes alternately and sometimes in conjunction with the aleuriospore racemes on the aerial hyphae, hyaline, smooth, septate at the base, usually simple, occasionally branched once or twice at a height of approximately 15 μ, somewhat awl-shaped, measuring approximately 20–22 × 2.8–3.3 μ. *Phialospores* produced in a single chain which sometimes slimes down into a ball-like mass; elliptic, hyaline, thin-walled, smooth, measuring 2.8–4.2 × 1.4–2.8 μ. *Sterile hairs* borne individually or in tufts on the creeping, aerial hyphae; rough, encrusted, olive-grey to brownish, usually simple, septate, measuring 180–240 × 2.8–3.9 μ, widening at the base to 4.2 μ; present in all collections from natural substrata, but not always found in culture.

Type locality: Belgium, on dung.

The colony on potato dextrose agar attains a growth of 2.0 to 4.3 cm in diameter in one week. At first composed of fine white mycelium, it gradually takes on a Tilleul-buff (Ridgway) color, becoming slightly zoned in small concentric areas of heavier and lighter growth, and showing orange color in reverse. The light pinkish-tan color is sometimes entirely covered over by the late appearance of olive-grey sterile hairs.

When the fungus is grown on corn stalks or on the leaves of *Phragmites communis* on mineral salts agar, the sterile hairs appear earlier, tend to grow more in tufts than individually, and quickly give a characteristic grey aspect to the culture; otherwise the growth is similar to that on potato dextrose agar.

Insofar as can be ascertained, *Botryotrichum piluliferum* is not represented in modern reports, but has previously been found on sacking in England (Wakefield, **1919**), on cheese in France (Loubiere, **1924**), and in soil in Canada (Bisby *et al.*, **1933**).

In a discussion of *Botryotrichum*, mention should be made of the species described by Saccardo (**1893**) as *B. lachnella*, which he considered different enough from *B. piluliferum* to place in a separate subgenus, *Peziotrichum*. This name was raised to generic rank by Lindau (**1900**) and has been maintained as a separate genus since. Petch (**1927**), discussing entomogenous fungi collected in Ceylon, presented a critical and detailed account of *Peziotrichum lachnella* and its relationship to *Botryotrichum piluliferum*, and concluded that *Botryotrichum piluliferum* is generically distinct from *Peziotrichum lachnella* because of the dark-colored hyphae of the latter. The similarity between original illustrations of *Botryotrichum* (*Peziotrichum*) *lachnella* (FIG. 1, H) and *Botryotrichum piluliferum* (FIG. 1, A) seems to indicate a close relationship. However, an examination of Saccardo's type of *Botryotrichum lachnella* reveals a growth composed of deep reddish brown, smooth, septate sterile hairs which are occasionally branched and rebranched, and which are always borne at the circumference of a circular, depressed acervulus; they measure 608–1040 × 3.9–5.2 μ. No spores were seen at this time. The growth is confined to small areas and shows no spreading mycelium. Its entire character is quite distinct from that of *B. piluliferum*.

The genus contains only one other species, *Botryotrichum atrogriseum* van Beyma (**1928**), found on fallen wood. A culture of this was deposited at the Centraalbureau voor Schimmelcultures, and has been examined in the present study. It has hyaline to dark mycelium which bears fruiting structures consisting of very dark (black in macroscopic examination), globose,

thick-walled aleuriospores, borne singly at the tip of extremely short, undifferentiated side branches which occasionally show some rebranching. No sterile hairs or phialides were found. On the basis of these characteristics, especially the dematiaceous aspect, this species is excluded from *Botryotrichum* at this time.

Botryotrichum as currently conceived is a monotypic genus, containing only the species *B. piluliferum*.

SUMMARY

Coccospora agricola Goddard is here reduced to synonymy under *Botryotrichum piluliferum* Sacc. and March. The species is shown to produce two types of spores, the predominating aleuriospores and the less prominent phialospores, the latter mentioned here for the first time. On the basis of this dual spore phenomenon, the description of the species is emended. Consideration is given to *Peziotrichum lachnella* Sacc., which had been considered a subgenus of *Botryotrichum;* the two are considered distinct genera in this study. The other species, *B. atrogriseum* van Beyma, is here excluded from the genus because of its dematiaceous character and its lack of sterile hairs and phialides.

ACKNOWLEDGMENTS

I am indebted to the late Dr. Samuel C. Damon for his very kind interest and suggestions in the work under consideration; to the late Dr. W. L. White of the Farlow Herbarium at Harvard University for the loan of authentic material of *Botryotrichum piluliferum* Sacc. and March. (Rabenhorst No. *3992*); and to Prof. Capelleti of Istituto Orto Botanico dell'Università, Padova, Italy, for the type specimen of *Botryotrichum lachnella* Sacc.

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TAXONOMIC STUDIES IN THE HYDNACEAE WITH REFERENCE TO THEIR HYPHAL SYSTEMS [1]

M. A. Ragab

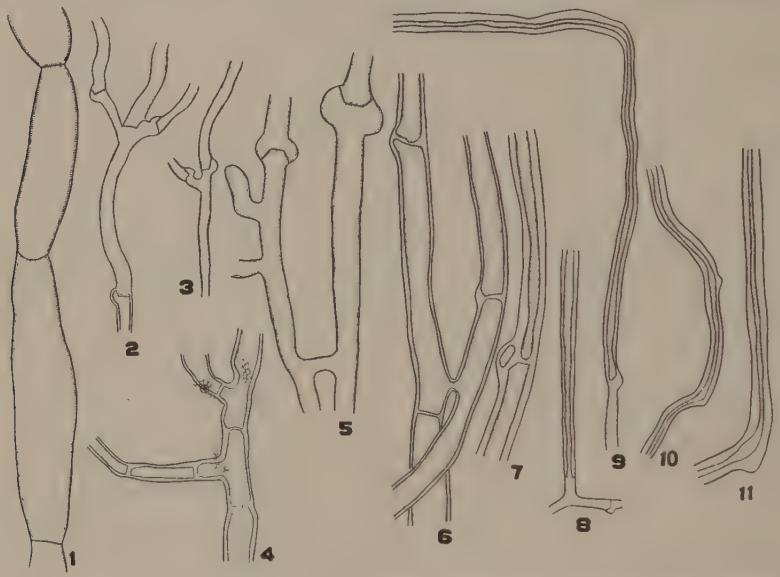
(with 11 figures)

In the early systematic arrangement of the higher fungi proposed by Fries, emphasis was given to gross characters which are most easily seen but which are frequently of less value in showing relationship among the groups than other, less conspicuous features. The arrangement based on the shape and appearance of the hymenium, which served Fries, has been subjected to modification and well-based criticism. Fries's system, which emphasized hymenial configuration and which disregarded the more fundamental characters of the basidia stressed recently by Rogers (10, 11), and the more detailed morphology of the hyphae pointed out by Corner (2, 3, 4) and Cunningham (5), has often resulted in unnecessary duplication of names.

Great diversity of opinion exists, not only with regard to the basis of generic limitations in the Hydnaceae but as to the value of various characters which have been used in classification. Emphasis has been placed upon diagnostic characters such as the shape of the fruit body, the character of spines, the presence or absence of sterile bodies such as cystidia and setae, spore color, and consistency of fructification. The consistency characters of the fructification such as tough-fibrous, coriaceous, and fleshy, may be used with various meanings. Toughness may be caused by the presence of skeletal hyphae as in the genera *Gloiodon, Asterodon, Auriscalpium* and most species of *Steccherinum*. The genus *Hydnellum* is characterized by a tough-fibrous structure which is

[1] Portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Botany at the State University of Iowa, Iowa City, Iowa, under the direction of Dr. G. W. Martin.

apparently due to moderately thick-walled, not inflated, generative hyphae, rather densely packed and with a tendency to agglutinate into strands in places. The genera *Hydnum* and *Dentinum* have a fleshy texture which may be caused by the thin-



All figures were drawn from KOH-phloxine preparations with camera lucida at × 1680 and reduced in reproduction to × 500.

FIGS. 1–7. Generative hyphae. 1. *Dentinum repandum*, inflated hypha. 2. *Odontia arguta*, profusely branched hyphae. 3. *Oxydontia Himantia*, finely incrusted hyphae. 4. *Odontia laxa*, thick-walled hyphae. 5. *Steccherinum pulcherrimum*, hyphae with double clamp-connections and H-connection. 6. *Hericium Erinaceus*, thick-walled hyphae with clamp-connections and H-connection. 7. *Hericium Erinaceus*, thick-walled hyphae with clamp-connection. FIGS. 8, 9. Skeletal hyphae at their origin from generative hyphae. 8. *Odontia stipata*. 9. *Oxydontia macrodon*. FIGS. 10, 11. Skeletal hyphae. 10. *Gloiodon strigosus*. 11. *Steccherrinum ochraceum*.

walled, inflated generative hyphae, loosely united and forming an open tissue.

Any student who has devoted considerable attention to taxonomic study of the Hydnaceae will recognize that little or no attention has been given to the character of the hyphal elements in delimiting genera and species. One object of this study has been to attempt to interpret the structure of the basidiocarp of the

Hydnaceae in the light of the new morphological concepts applied by Corner to the related groups of pore and clavarioid fungi. In the present paper all recognized genera of North America, and species found in Iowa as well as other places have been used for the study. Such a study, supplemented by an examination of gross characters, will furnish data which may be useful in presenting an exact definition of genera and species.

Miller & Boyle (**9**) recognize sixteen genera. The genus *Caldesiella* is treated by them in the Hydnaceae but they pointed out its close relation to *Tomentella*. Rogers (**10, 12**) pointed out that *Caldesiella* and *Tomentella* are probably not separable from each other; the two must be regarded, not as members of two distinct families, Hydnaceae and Thelephoraceae, but as only slightly different aspects of a single genus. Hyphal analysis of *Caldesiella ferruginosa* and several species of *Tomentella* has clearly revealed close affinity between them and Rogers seems justified in his conclusion. The proposal of Rogers to conserve *Tomentella* Pat. against *Caldesiella* Sacc., supported by the Nomenclature Committee of the British Mycological Society (**13**) and by Donk (**6**), was approved by the Stockholm Congress.

Coker and Beers' (**1**) recognition of *Pseudohydnum* Rick is unfortunate. It should be noted that the generic name *Pseudohydnum* Rick 1904 is antedated by *Pseudohydnum* Karsten 1868. There is no doubt that *Pseudohydnum* Rick is a later homonym of *Pseudohydnum* Karst. in the Tremellaceae, and must be replaced by *Hydnodon* Banker 1913. Martin (**7, 8**) revived Karsten's genus and is using it in place of the later *Tremellodon*.

As a result of this study it has been found that the majority of the species examined are monomitic. The genera *Radulum, Grandinia, Hydnum, Hydnellum, Hydnodon, Dentinum,* and *Hericium* are monomitic. The monotypic genera *Asterodon, Auriscalpium,* and *Gloiodon* are dimitic. The species of *Steccherinum, Oxydontia,* and *Mucronella* may be either monomitic or dimitic. The majority of the species of the genus *Odontia* are monomitic; of twenty species examined only three have a dimitic system of hyphae. On the other hand, of eight species of *Steccherinum* only one is monomitic. In *Oxydontia,* of six species

studied, only one is dimitic. In *Mucronella*, of three species studied, only one is dimitic.

It is apparent that some of these genera will probably have to be divided, since it is doubtful whether both monomitic and dimitic species should be included in *Steccherinum, Odontia, Oxydontia,* and *Mucronella.* Corner (4) has already transferred dimitic species of *Mucronella* to *Deflexula.* However, it seems wiser at the present time to expand the concept of these genera so as to include these species, at least until tropical specimens are studied.

Two hyphal systems are present in the family, mononitic, and dimitic. They may be composed of two hyphal types generative and skeletal. The terms monomitic, dimitic, generative and skeletal are used in the sense of Corner.

In the recognition of genera, a complex of characters rather than a single character is considered. The writer's present knowledge of the Hydnaceae seems to warrant the recognition of fourteen genera. The genus *Hydnellum* is emended so as to include the species formerly included in the genus *Phellodon.* Both *Hydnum albonigrum* Peck and *Hydnum amicum* Quél. agree in hyphal structure with *Hydnellum velutinum* (Fries) Karst. The major differences are in spore color and markings and in superficial external characters, none of which are regarded as sufficient to justify generic segregation. Both have been referred to both *Phellodon* and *Calodon,* neither genus, however, valid against *Hydnellum* as here emended. It is necessary, therefore, to propose as new combinations **Hydnellum alboniger** (Peck) comb. nov. and **Hydnellum amicum** (Quél.) comb. nov.

KEY TO THE GENERA OF THE HYDNACEAE

a. Subiculum lacking or represented by a fugacious mycelium; spines simple or branched, arising directly from substratum; monomitic or dimitic; on wood and bark.....I. *Mucronella*
a. Subiculum present; trama dark or pale.....b
b. Fructification resupinate, reflexed to pileate and laterally sessile, or stipitate; monomitic or dimitic; on wood, or cones of conifers....c
b. Fructification pileate and stipitate; monomitic; on the ground.....k
c. Trama dark; dimitic.....d
c. Trama pale; monomitic or dimitic.....e
d. Resupinate, floccose; stellate setae present; clamp-connections lacking, spores hyaline, smooth; on wood.....II. *Asterodon*

d. Resupinate or reflexed to pileate and laterally sessile; spines borne on tough profusely branched processes partially submerged in a brownish tomentum; clamp-connections present; spores hyaline, faintly roughened; on wood.....III. *Gloiodon*

e. Resupinate, thin; floccose, crustaceous, ceraceous, or subcoriaceous.....f

e. Reflexed, sessile or pileate and stipitate, rarely resupinate; fleshy, tough, to coriaceous.....i

f. Cystidia present; texture variable; monomitic or dimitic; clamp-connections present or absent; skeletal hyphae unbranched; spores smooth or echinulate; on wood.....IV. *Odontia*

f. Cystidia absent.....g

g. Ceraceous; teeth blunt, often deformed and irregularly scattered; rarely reflexed; monomitic; on wood.....V. *Radulum*

g. Texture variable; strictly resupinate; teeth hemispherical to cylindrical or subulate.....h

h. Teeth in the form of short, fragile, hemispherical or cylindrical warts; monomitic; on wood.....VI. *Grandinia*

h. Teeth or spines typically long, conspicuous, slender, usually terete; monomitic or dimitic; on wood.....VII. *Oxydontia*

i. Fructification abundantly branched or pulvinate, fleshy-fibrous; monomitic; generative hyphae often very thick-walled; gloeocystidia usually present; on wood.....VIII. *Hericium*

i. Fructification not branched nor pulvinate; subfleshy to coriaceous; dimitic, rarely monomitic; cystidia present or absent.....j

j. Fructification laterally stipitate, stipe long, slender; pileus coriaceous; dimitic; cystidia present; spores hyaline, minutely roughened; on pine cones.....IX. *Auriscalpium*

j. Fructification reflexed to laterally stipitate (rarely resupinate), subfleshy to coriaceous; often dimitic, rarely monomitic; cystidia present or absent; spores hyaline, smooth; on wood..X. *Steccherinum*

k. Fructification fleshy, fragile; hyphae inflated, thin-walled; spores hyaline, smooth.....XI. *Dentinum*

k. Fructification not fragile; spores subhyaline to distinctly colored, echinulate or tuberculate.....l

l. Subgelatinous; hyphae thin-walled, not inflated; spores coral pink, tuberculate.....XII. *Hydnodon*

l. Fleshy-tough to fibrous.....m

m. Fleshy; hyphae thin-walled, inflated, clamp-connections present or absent; spores brown, tuberculate.....XIII. *Hydnum*

m. Fibrous-tough; hyphae rather thick-walled, not inflated; clamp-connections not seen; spores either brown and tuberculate or subhyaline and echinulate.....XIV. *Hydnellum*

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ABORTIVE "PERITHECIAL" PRODUCTION BY PHIALOPHORA VERRUCOSA

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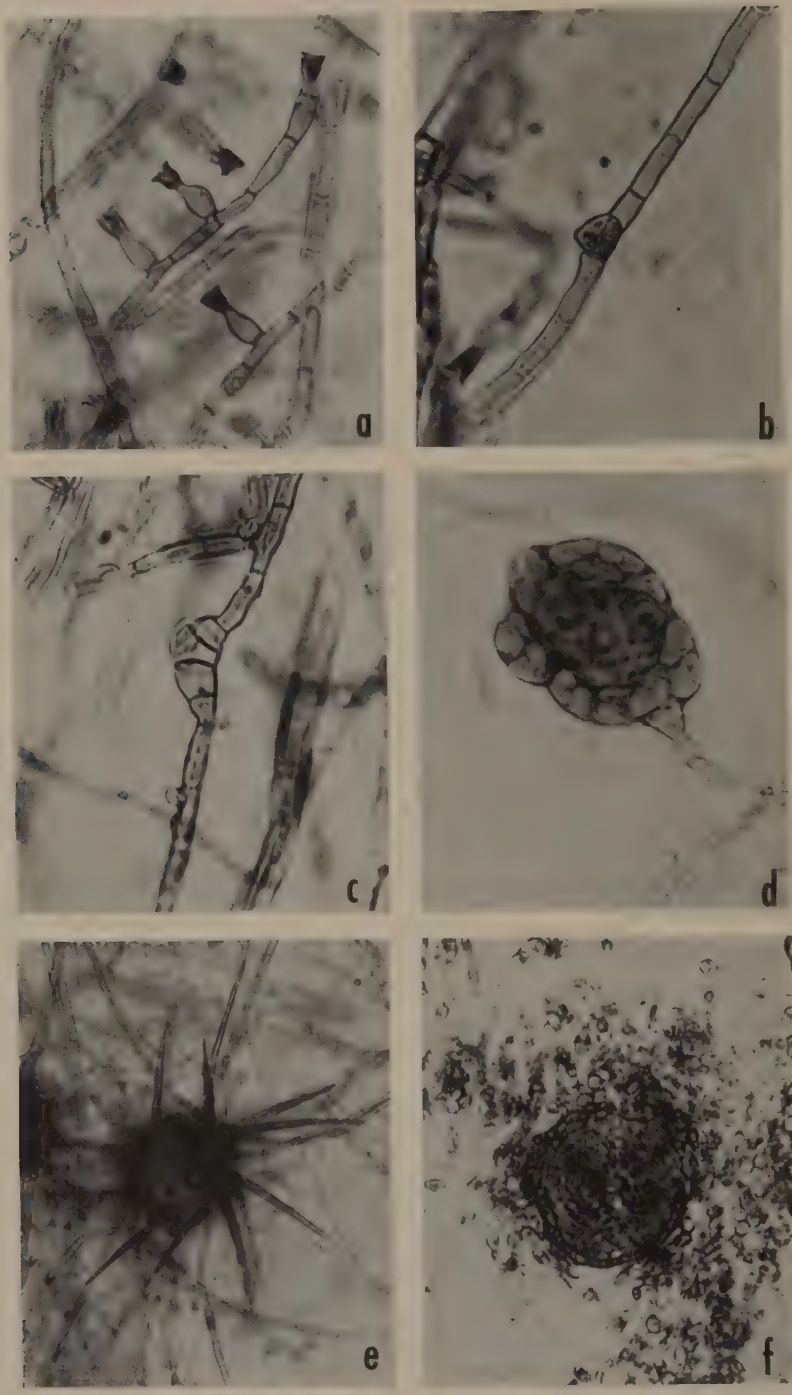
(with 2 figures)

Reproduction in the majority of the fungi pathogenic to man and lower animals is by asexual means. Only two species, *Allescheria boydii* Shear, 1922 (7), and *Piedraia hortai* (Brumpt) Fonseca & Area Leao, 1928 (3), are known to possess a perfect stage. Both are classified among the ascomycetes.

Despite the rarity of sexual reproduction among fungi causing animal diseases, it is possible that under certain natural or experimental conditions any or all of these imperfect organisms may become capable of producing sexual spores. Indeed such a phenomenon was observed by Emmons (2) to have occurred spontaneously in an isolate of *Monosporium apiospermum* Saccardo, 1911 (5, 6), long maintained in culture. This organism produced perithecia and ascospores referable to *A. boydii.* A similar event was thought to have occurred in our laboratory when a student drew attention to some unusual structures resembling perithecia in a teased mount of *Phialophora verrucosa.*

P. verrucosa is a well-known agent of chromoblastomycosis among humans (1) and is one of the fungi responsible for the blueing of timber (4). Reproduction in this "fungus imperfectus" is by means of unicellular spores, borne semi-endogenously on flask-shaped conidiophores (Fig. 1a). The observation of large dark spherical bodies bearing setae (Fig. 1e) in a large series of cultures of one stock strain was thought to indicate another means of reproduction. However, despite their morphologic similarity to perithecia, these structures have all proven to be infertile. Repeated attempts to cross this isolate with several other strains of this fungus maintained in the laboratory, and the use of various types of natural and semi-synthetic substrates to induce the complete development of the "perithecia" have all been fruitless.


a
b
c
d
e
f

Despite this failure, it has been thought worth while to describe briefly the development of what appears to be abortive perithecia in *P. verrucosa*.[1] This is done with the hope that other observers may be stimulated to search for similar developments in their cultures of this organism and perhaps observe or bring about completion of the cycle.

The "perithecial" initials arose in an intercalary or terminal position in the mycelium (FIG. 1 b, d). By multiplication of the primary cell, a solid, multicellular, more or less spherical body

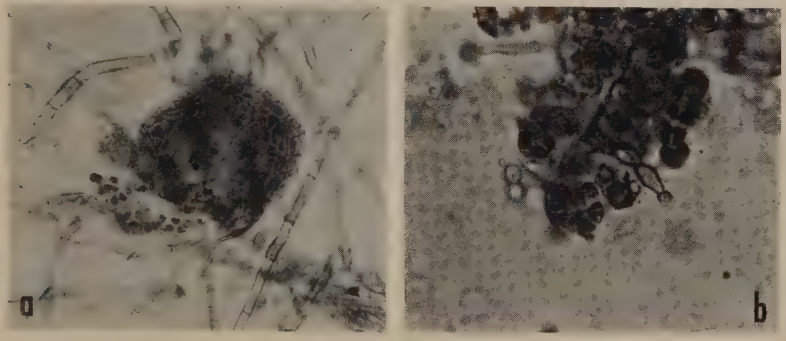


FIG. 2. a. Crushed "perithecium" showing escaping contents, × 160; b. Group of pseudoparenchymatous cells from interior of a "perithecium" (aceto-carmine mount), × 160.

was developed (FIG. 1, d). As this structure increased in size dematiaceous filaments were formed on its surface (FIG. 1 e). These setae were attenuated at the tip and were composed of one or several cells. They measured from 20–60 μ in length and averaged 4 μ in diameter. The "perithecia" at their maximum size were composed of a mass of pseudoparenchymatous cells surrounded by a peridium, one cell layer thick (FIG. 1 f), and ranged from 20–90 μ in diameter.

[1] This particular isolate of *P. verrucosa* was received from Tulane University, but unfortunately a history of its ultimate source could not be furnished.

FIG. 1. a. Conidiophores of *P. verrucosa,* × 490; b. Intercalary development of "perithecial" initial, × 694; c. Early stage of cellular multiplication, × 490; d. Multi-celled terminal "perithecial" initial, × 490; e. "Perithecium" with setae, × 190; f. Cross section of fixed and stained "perithecium" showing peridium and undifferentiated interior, × 245.

No trace of ascogonial initials were observed in fixed and stained preparations. When pressure was applied to the perithecia, the peridium ruptured readily permitting groups of pseudoparenchymatous cells to escape (FIG. 2 a, b).

Several other isolates of *P. verrucosa* were studied along with the Tulane culture but none produced these interesting structures.

To date, this is the extent of our observations. However, nutritional and physiological studies currently are under way to determine whether the conditions necessary to complete this apparent attempt at sexual reproduction can be discovered.

The authors are indebted to Mr. John P. Gust of the Audio-Visual Services Section for the photomicrographs.

SUMMARY

1. In a culture of *Phialophora verrucosa,* the development of perithecial-like structures was observed.

2. Attempts to induce completion of their development ended in failure.

3. A description of the developmental states of these bodies is presented.

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HUMICOLA GRISEA, A SOIL-INHABITING, CELLULOLYTIC HYPHOMYCETE

W. Lawrence White[1] and Mary H. Downing[2]

This is the second paper[3] of a proposed series dealing with a group of Hyphomycetous soil fungi characterized morphologically by the copious production of globose, thick-walled aleuriospores as the principal spore type, and physiologically by the fact that they are in the main among the strongest of the cellulose-decomposing fungi. The present installment is confined to a consideration of *Humicola grisea.*

HISTORICAL AND TAXONOMIC SIGNIFICANCE OF THE SPECIES

The genus *Humicola* was established by Traaen (**1914**) on two species which he isolated repeatedly from Norwegian soils. His first and most frequently isolated species, *H. fuscoatra,* was characterized by spores on the order of 6–9–12 μ in diameter with walls of medium thickness and of a very dilute color; his second, *H. grisea,* by larger spores, 12–17 μ in diameter, with thicker and much more deeply colored walls. A modern investigator examining the respective type cultures, which have been maintained at the Centraalbureau since about 1912, might raise a question as to whether or not they merely represent extremes of variation within a single species. However, an adequate number of cultures has now been accumulated to demonstrate beyond all doubt that there actually are two distinct species as indicated by Traaen. The first species of Traaen, i.e., *H. fuscoatra,* is to be regarded as the generic type.

Traaen's *H. grisea* took on a peculiar and interesting significance taxonomically when Mason (**1933**, p. 56) speculated that it

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[2] Pioneering Research Laboratories, U. S. Army Quartermaster Corps, Philadelphia, Pa.

[3] The first was published in *Mycologia* **43**: 645–657. 1951.

(under the name *Monotospora Daleae* Mason) was synonymous with *Monotospora toruloides* Corda, the single species upon which Corda (**1837**) had founded the genus *Monotospora*. This being true, it would of course follow that *Humicola* Traaen was a synonym of *Monotospora* Corda. Actually, Mason, a few years later (**1941**, p. 113) discovered the paper of Traaen and expressed the opinion that Traaen's *Humicola* should be referred to synonymy under *Monotospora* Corda but not under what he called "*Monotospora* Sacc." Some years after the work of Corda, Saccardo (**1886**) had taken up the generic name *Monotospora* Corda in a sense that excluded Corda's *Monotospora toruloides*. Various writers have commented upon the taxonomic complications involved. Various ways have been suggested for dealing with the nomenclatural problem. That which has been basic is Mason's proposal to refer *Humicola* Traaen to synonymy under *Monotospora* Corda and to use "*Monotospora* Sacc." for the several species, probably of a miscellaneous nature, which Saccardo transferred to his (Saccardo's) *Monotospora* Corda. What Mason calls "*Monotospora* Sacc." would then, according to Mason, be conserved against *Monotospora* Corda leaving the way open for the acceptance of *Humicola* as a good genus.

Diehl (**1949**, p. 279) summed up Mason's point of view, and also his own, in the following words: "He [Mason] pointed out that, if *Monotospora* of Saccardo should be conserved against Corda's prior name, *Humicola* as a synonym of the discarded *Monotospora* of Corda would be legitimate with *H. fuscoatra* Traaen as the type species. Because of its convenience this should be a welcome taxonomic disposition of that hitherto debated form-genus"

The present paper deals with *Humicola*, and no detailed analysis of the early history of *Monotospora* will be attempted; nor would it be advisable on our part until we had gained a better knowledge than we now have of the species placed in *Monotospora* by Saccardo. Brief comment appears desirable, however, in order to explain our acceptance of *Humicola*. Rogers (**1949**, p. 459; **1950**, p. 28) has, in our opinion, dealt effectively with "*Monotospora* Sacc." in stating that there simply is no such thing. Must *Humicola*

then be relegated to synonymy under *Monotospora* Corda? If Mason's contention that *Humicola grisea* is synonymous with *Monotospora toruloides* Corda is correct, the answer obviously is 'yes.' We prefer, however, to move in what appears to be a current drift toward the acceptance of *Humicola* and we believe there are reasons for doing so that are more basic than any heretofore suggested.

There is no overwhelming evidence that Corda's *Monotospora toruloides* is the same as Traaen's *Humicola grisea*. We submit that Corda's drawing could just as well represent *Papularia sphaerosperma* (Pers.) Höhn. Corda's organism formed a black effuse growth on a dead monocotyledonous substratum. This is the characteristic substratum and habit of growth for the *Papularia*, but not for *Humicola grisea*. The latter, insofar as has been demonstrated, is strictly a soil species or an inhabitant of humus or decaying wood in the soil. It has never been found under natural conditions in the form of a sporulating, hyphomycetous turf on the surface of any plant debris.

The microscopic morphology depicted by Corda fits the *Papularia* at least as well as the *Humicola*. The proportionate thickness of wall to size of spore by Corda is approximately correct for that of the lenticular spores of *Papularia* as they are commonly seen in face view in microscope mounts and is too great for *Humicola*. There is evidence of the lenticular shape in at least one of the spores drawn by Corda.

At any rate the two species with which the writers are principally involved, *grisea* and *fuscoatra*, are now in *Humicola*. Neither of them has a legitimate combination in *Monotospora*. Transferring them to *Monotospora* would require changes in nomenclature and add to synonymy. The "burden of proof" should fall upon the individual who makes the change. In this case we can offer neither proof of necessity for such transfers nor evidence of desirability from the point of view of phylogeny or nomenclatural stability.

Practically all the records, or possible records, of this species have come from studies in soil microbiology. It is of interest to note that of the many cultures of *Humicola* that originated from

industrial and military materials in the tropical deterioration program all were *H. fuscoatra*. This is in accord with the findings of Diehl (**1949**). The present writers believe that they have seen all of these cultures regardless of the laboratory in which the isolations were made.

The earliest acceptable records of *H. grisea* are those of Jensen (**1912**) and Dale (**1912**). This was just prior to Traaen's establishing the genus *Humicola*. Jensen's isolates were wrongly assumed by him to be identical with an organism that had been described by Morgan in 1895 as *Monotospora nigra* Morgan, and Dale's was wrongly presented (Dale **1912**; Smith and Ramsbottom **1912**) as *Basisporium gallarum* Moll.

Mason is to be credited with the sorting out of the cultures involved in the early history of *Humicola grisea*.

SYNONYMY

HUMICOLA GRISEA Traaen, Nyt. Mag. Nat. **52**: 34. 1914.

Monotospora Daleae Mason, Annotated account of fungi received at the Imperial Mycological Institute, List II (Fascicle 2), p. 50, 1933.

Melanogone puccinioides Wollenw. and Richter, Zentralbl. fur Bakt. Abt. 2. **90**: 76. 1934.

GEOGRAPHIC DISTRIBUTION, HABITAT, MATERIAL EXAMINED

Massachusetts: Harvard Forest *I–1–2, I–1–17b,* and *I–6–2,* isol. at Harvard Unit., 12 Oct. 1949 by Frank L. Raymond from well-drained sandy loam at point 4 inches below surface of mineral soil in 26 yr. old pine-spruce plantation, Harvard Forest, Petersham, Mass., Plot 26N., Prospect Hill Compartment I; Harvard Forest *II–1–20, II–7–9,* and *II–7–10,* isol. at Harvard Forest, 6 July 1950 by Frank L. Raymond from loam soil taken at line of demarcation between organic matter and mineral soil in same plot as above.

New York: Said by Jensen (**1912**) to be common in cultivated soil at Ithaca and isol. commonly in summer, winter, and fall. Jensen made the combination *Mycogone nigra* (Morgan) Jensen

on the erroneous assumption that he was dealing with *Monotospora nigra* Morgan. The latter (type specimen loaned us by Dr. G. W. Martin) is a very different thing. Jensen's description is almost certainly based on *Humicola grisea* but the specimen he cites is lost (Mason, **1933**, p. 54; also personal communication from G. C. Kent, June 1951). Whether or not all of Jensen's isolates should be referred to *H. grisea* is, of course, unproven.

New Jersey: Waksman (**1916**) reported an isolate which he questionably labeled *Basisporium gallarum* Moll. from iron (48 per cent) soil taken at a depth of 8–10 in. in a peach orchard at Keyport. This would appear from his description to be *H. grisea*. It did not appear in several other soils. The isolate proved to be strongly active in decomposing a chemically modified cellulose. Waksman mentioned isolates of *Humicola* sp. in several additional papers but their specific identity is unknown. The writers have seen only one isolate of *Humicola* by Waksman, his No. *63*, which is *H. fuscoatra* as indicated by Mason and Diehl. No data are available concerning its origin (personal communication from Waksman).

Manitoba: ATCC *6725* (QM *542*), a culture sent (as *Monotospora Daleae*) by Dr. J. E. Machacek, then of the Dominion Laboratory of Plant Pathology, Winnipeg, to the Am. Type Cult. Col. in 1938 without detailed data. This is undoubtedly confirmatory of the reports by Bisby, James and Timonin **1933**, p. 266; Bisby, Buller and Dearness **1933**, p. 100; Bisby **1938**, p. 121, that the species is common in soils of grasslands and wheatfields in Manitoba "but not found in other soils."

Saskatchewan: Isolated from roots of wheat from Indian Head (Bisby **1938**, p. 121).

Finland: Based on the work of Vartiovaara (**1935**).

Poland: Based on work of Felsz-Karnicka (**1935**). Cultures not seen by us.

Norway: Culture obtained from C.B.S. and accessioned here as QM *993*, isolated from soil in Norway by Traaen (see Traaen **1914**), regarded as the type of the species. Traaen, using filter paper as an isolation medium, obtained cultures of the species from 17 samples of substrate from such habitats as decaying tree

trunks, at least some of which were coniferous, bits of leaves from various hardwoods, and samples of soil from near surface of meadow, muck, an anthill, graveyard, brook bed and potato field. It was especially common in field and meadow soil and rotten tree trunks.

Germany: Based on work of Wollenweber and Richter (**1934**). Organism made by them the basis of a new genus and species, *Melanogone puccinioides,* and later properly referred to *Humicola* by Mason (**1941**). Said to be an inhabitant of forest litter, decaying tree roots, and decaying structural timber at or just below ground level, specifically in roots of *Ulmus scabra-montana* killed by Dutch elm disease, forest litter of birch-pine and of spruce; not forming any conspicuous surface deposition of mold growth. A culture deposited at C.B.S. by Wollenweber was examined by us.

England: Culture obtained from C.B.S. and accessioned here as QM *992,* isolated by Dale from soil at Woburn, England, the soil having been "continuously manured for 38 years with sulphate of ammonia" and having acquired a "distinctly acid reaction." (See Dale **1912**, Smith and Ramsbottom **1912**, Mason **1933**, **1941**, Wakefield and Bisby **1941**.)

Ceylon: Culture cited by Mason (**1933**), from 'Adco,' C. H. Gadd *87.* Not examined by us.

Australia: Culture cited by Mason (**1933**), from soil, Melbourne, E. McLennan. Not examined by us.

CHARACTERISTICS OF THE SPECIES

Morphological. Humicola grisea is known only in pure culture, there being as yet no authentic record of its ever having been identified in the form of a superficial fruiting layer on natural substrata, which is characteristic of so many of the Dematiaceae. A possible exception is the fact that Wollenweber obtained aleuriospores and phialospores on naturally infected wood which he removed from its source and exposed to another set of conditions. On agar media growth is fairly rapid and aleuriospores are formed among the underlying aerial hyphae, quickly, and usually in some abundance. FIG. 1 shows duplicate plates of four isolates grown in parallel series under room conditions on potato dextrose agar

(per liter: 20 gm dext., 20 gm agar, water from 400 gm boiled potatoes). It will be noted that the heavy, fairly compact, cottony mat in eleven days nearly reached the margin of the 9 cm petri dishes.

The four strains shown in FIG. 1 were used for comparison of colony characteristic mainly because they were the four most conveniently available at the time. Incidentally, they represent wide variation in age of isolate and geographic range. (A) represents a culture isolated from soil in England at least 38 years ago; (B) was isolated from soil in Norway at least 36 years ago; (C) was obtained from soil at Petersham, Massachusetts 1 year before the photo was taken; (D) was taken from a Manitoba soil about 20 years ago. These strains exhibit what most workers would consider to be a remarkable stability in culture and an equally remarkable uniformity among strains of a species. The four strains show no differences that could be put into words with the exception of (A) where the colonies exhibit a dark central area, due largely, if not entirely, to the formation of globules of liquid on and within the hyphal mat.

Growth is at first nearly white, then a very pale gray, gradually deepening and with age reaching a fairly dark gray. The color is mostly in the dark aleuriospores which develop among the hyphae. The pedicels are also noticeably dark as seen under the microscope, while the hyphae, though hyaline as seen individually, may contain a certain amount of pigment as seen in mass.

The aleuriospores (FIG. 2, A–D, and F–J) are smooth, dark, single-celled, typically globose, with an occasional one varying to obovoid or pyriform. The walls are of substantial thickness. The spores fundamentally are borne singly but it is not unusual to find two (FIG. 2, A, F) or three clinging together. Certain writers have been led to make reference to two-celled spores through the interpretation of an inflated hyphal cell adjacent to the spore (FIG. 2, G left of center, F, and I) as part of the spore. Most of the spores fall within the range of 12 to 15 μ in diameter with any strain exhibiting an occasional spore under and over this range. Detailed measurements have been made for five strains with the following averages for 70 spores:

FIG. 1. Four cultures of *Humicola grisea* grown in parallel series for comparison of gross colony characteristics: A. QM *992*, the culture iso-

1. QM *993,* Traaen's type from 6-weeks-old colony on potato dextrose (2 percent dext.) agar, mounted in water = 13.2 μ.

2. QM*992,* Dale's culture, grown in parallel series with the preceding, also mounted in water = 13.2 μ.

3. Harvard Forest *I–1–2,* in parallel series with the two preceding = 14 μ

4. Harvard Forest *II–1–20,* from a potato dextrose agar (2 percent dext.) stock slant of unknown age, probably from refrigerated storage = 12.8 μ.

5. ATCC *6725* (QM *542*), grown in parallel with 1–3 above = 12.4 μ.

These measurements agree closely with those recorded by Traaen, Bisby, and others, and are recorded only for the purpose of showing that there is no great amount of variation among strains.

The variations in microscopic characters described above are variations within a strain. No noteworthy variation has been found among strains in the course of routine examinations and comparison of photomicrographs. In accompanying plates the spores of the recent isolate Harvard Forest *I–1–2* (FIG. 2, G–J) may be compared with those of Traaen's type culture (FIG. 2, A–C).

Traaen, Wollenweber, and Mason all found phialospores and phialides in their cultures in addition to the aleuriospores. These were inconspicuous, however, and were encountered only under certain conditions. Traaen noted their development as a whitish covering on old colonies grown on filter paper and mineral salts. Mason found them in all the strains with which he worked, but only on potato dextrose agar, and in all but one isolate they were always in old cultures after the period of active aleuriospore formation. Wollenweber produced them on the surface of naturally

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lated by Dale prior to 1912 from soil in England; B. QM *993,* Traaen's type culture isolated from Norwegian soil prior to 1914; C. Harvard Forest *I–1–2* from soil at Harvard Forest, Petersham, Mass. in 1949; D. ATCC *6725* isolated from soil in Manitoba about 1930. Colonies started by transplanting from stock cultures a small piece of agar with fungus. Grown on potato dextrose agar (per liter: 20 gm dext., 20 gm agar, water from 400 gm boiled potatoes), 35 cc medium per dish, room conditions, 11 days. Magnif. approx. ½. Photo by Frank White.

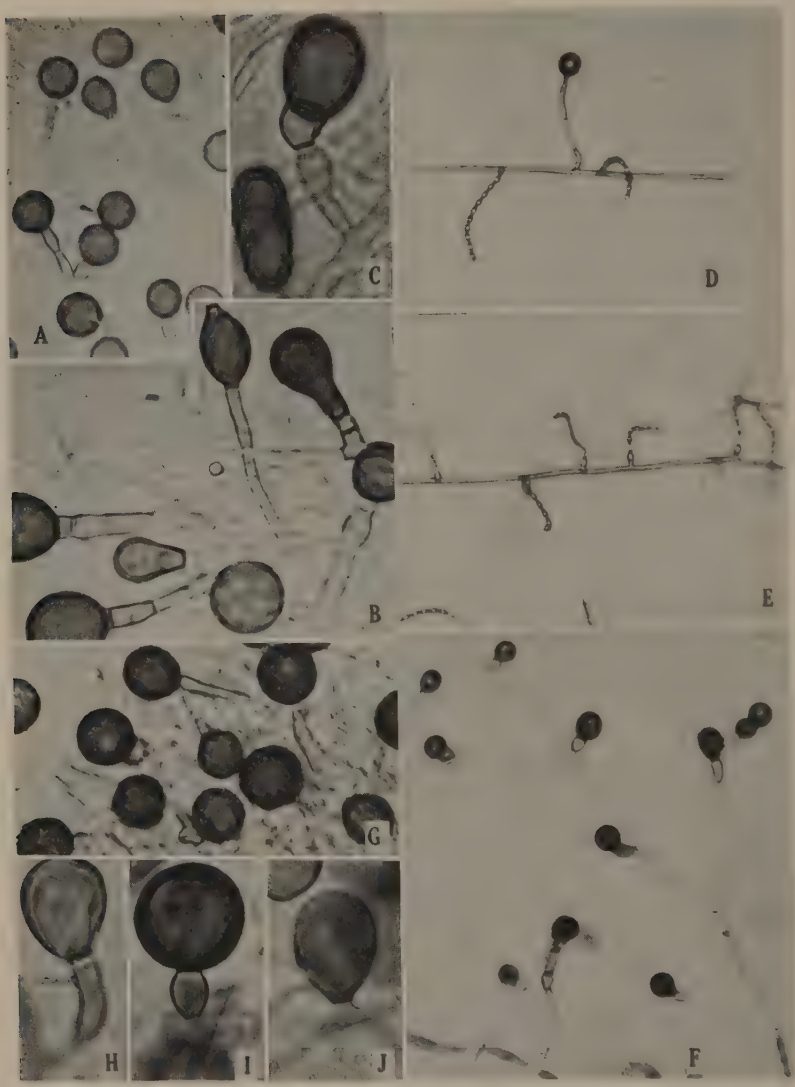

FIG. 2. Photomicrographs of *Humicola grisea*. A–F. QM *993* from plate cultures of Traaen's type: A, × 500, B, × 775, and C, × 1000, aleuriospores of the culture on peptone-glucose (0.5–1 percent) agar after 12 days under room conditions, mounted in lacto-phenol, unstained; A and B from growing culture, photomicrograph by Leo Kaplan; C from the same culture after it had been dried down as an herbarium specimen, photomicrograph by Paul Brown. D, E, F, all × 260. Photomicrographs by the junior author, taken directly from a culture on mineral salts agar containing fragments of corn stalk and leaves of *Phragmites communis* after 12 days at

infested wood, possibly by placing the pieces in a moist chamber. The phialides appear as single, elongate, tapering cells about 8–16 × 2–3 μ, seated laterally on a hypha. They extrude small, hyaline, obovoid spores about 3 × 2 μ, in chains or in balls. In the present study phialides have again been demonstrated in QM *993*, a culture from Traaen's type grown on fragments of corn stalk and on leaves of *Phragmites communis* in mineral salt agar (30° C, 12 days) (FIG. 2, D, E). Under the same conditions, no phialides were found in the six other cultures examined.

Physiological. The species appears to be characterized by a physiological stability comparable to the morphological. This at least is true of its cellulolytic ability. In a previous paper (see footnote, first page), a table was presented comparing the cellulolytic activity of numerous isolates of four allied species (*Humicola grisea, H. fuscoatra, Coccospora agricola,* and *Monotospora lanuginosa*). With the exception of *M. lanuginosa,* all exhibited strong cellulolytic properties.

The cellulolytic activity of these isolates did not appear to be affected by the length of time that they had been maintained on artificial media containing a sugar as the carbon source. The minimum periods under which the isolates under test were kept on such media:

QM *992*—38 years
QM *993*—36 years
ATCC *6725* = QM *542*—20 years
Harvard Forest *I–1–2* = QM *994*— 1 year
Harvard Forest *I–6–2* = QM *995*— 1 year
Harvard Forest *I–1–17b* = QM *996*— 1 year

30° C, no mount; D shows aleuriospore and phialospores produced from same basal hypha; E illustrates phialospore production, and F shows branching hyphae bearing aleuriospores. G–J. Photomicrographs by Paul Brown of aleuriospores from Harvard Forest *I–1–2* culture: G, × 500. From potato dextrose agar mounted in lacto-phenol, unstained; H, × 1000. Spore on an elongate, cylindric hyphal branch from an unknown medium, mounted in lacto-phenol, stained with fast green; I, × 1000. Spore seated on an inflated hyphal cell, mounted in lacto-phenol, stained with cotton blue; J, × 1000. Spore originating directly from the side of a hypha, mounted in lacto-phenol, stained with fast green.

In an attempt to round out the present study, excerpts from Traaen's work (1914) are presented covering the conditions for growth:

Temperature: 5–30° C; optimum 20–25° C.

Carbon Sources: Good: Glucose, sucrose, fructose, inulin, starch, xylan, pectin. Poor: Glycerol, maltose, mannitol.

Nitrogen Sources: Good: NH_4NO_3, KNO_3, $(NH_4)_2HPO_4$, alanine, leucine, tyrosine, urea, glycocoll, arginine, guanidine, nucleic acid, sodium humate. Poor: NH_4Cl, $(NH_4)_2SO_4$, creatine.

SUMMARY

1. *Humicola grisea* is a member of the Dematiaceae characterized mainly by the copious production on agar media of dark, globose, thick-walled aleuriospores 12–15 μ in diameter, and by its strong cellulose-decomposing capacity in pure culture tests.

2. It is an inhabitant of cultivated and forest soils and of decaying wood and other coniferous and hardwood forest debris in or on the soil, and is not known to form any surface deposition of mold growth in nature such as is characteristic of many of the Dematiaceae. Available data indicate occurrence in a great range of soil types, especially those rich in organic matter.

3. It has been isolated from widely separated parts of the world but mainly from parts of North America and Europe having cold winters. It appears to be common where adequate studies have been made.

4. Strains of the species exhibit a remarkable stability and uniformity in pure culture.

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A NEW SPECIES OF HYSTEROGRAPHIUM [1]

J. G. Brown

(with 2 figures)

The saprophytic ascomycete for which a name is proposed in this brief paper is a wood-destroying fungus. In general, fungi on such substrata are not numerous in the dry air of Arizona at the lower altitudes. Although fence-posts and other timbers, as well as natural woody debris in contact with the soil, are frequently found to be more or less decomposed, examination usually discloses that destruction is mainly the work of termites rather than solely that of fungi. The subject of this paper, an exception to the rule, is a fungus collected on the mesa near Tucson by a graduate student and brought to the laboratory for identification.

The fungus referred to is a fairly common inhabitant of the surface of decorticated trunks and branches of the cholla (pronounced choya) cactus, *Opuntia fulgida* Engelmann. There, the hysterothecia and other forms of fruiting bodies are commonly lined up in parallel rows (Fig. 1). With its red epithecia, the fungus reminds one at first glance of the "pulchra species," *Lophodermium versicolor* Wahlberg, a species sketchily described by Rabenhorst (1), from which it otherwise fundamentally differs.

Perithecia of the fungus are erumpent-superficial, sessile, nonstriated, and vary considerably in form and size. They are mostly oblong to ovoid, navicular (Fig. 2A, a, d), of the linear hysterothecial type (Fig. 2A, a, e), less often angular (Fig. 2A, f), or even hemispherical (Fig. 2A, b, e); their lips are tumid, bounding a slit-shaped to circular ostiole, depending upon the perithecial form, and exposing an epithecium Hay's Russet in color (2). They are coriaceous to carbonous, uniformly black in color excepting the epithecium.

In size the hysterothecia average (30 measured) 1.7×0.64

[1] Arizona Agr. Exp. Sta. Technical Paper No. 326.

FIG. 1. Perithecia of *Hysterographium opuntiae* on a piece of decorticated wood of *Opuntia fulgida* Engelmann. ×2½.

mm; the longest of 55 was 5 mm and the shortest 0.50 mm; the broadest of the most common oblong and navicular types was 1.0 mm, the narrowest 0.88 mm. These measurements apply to the freshly collected specimens. With age and consequent drying, the perithecia shrink, a condition that should be taken into account in measuring old herbarium specimens.

The asci (FIG. 2C, n–r) are clavate, straight to curved. The degree of curvature appears to depend upon the distance of the ascus inward from the ostiole. In general, the asci are 3–8-spored,

the latter predominating, but an occasional ascus with only one or two ascospores may be present (FIG. 2C, o); they are mostly obliquely monostichous. Asci varied in length from 85 to 160 μ; in breadth they are 12.5 to 16 μ.

The ascospores (FIG. 2C, D) are mostly ellipsoid to ovoid, predominantly muricate with 3–5 transepta and 1–2 longisepta,

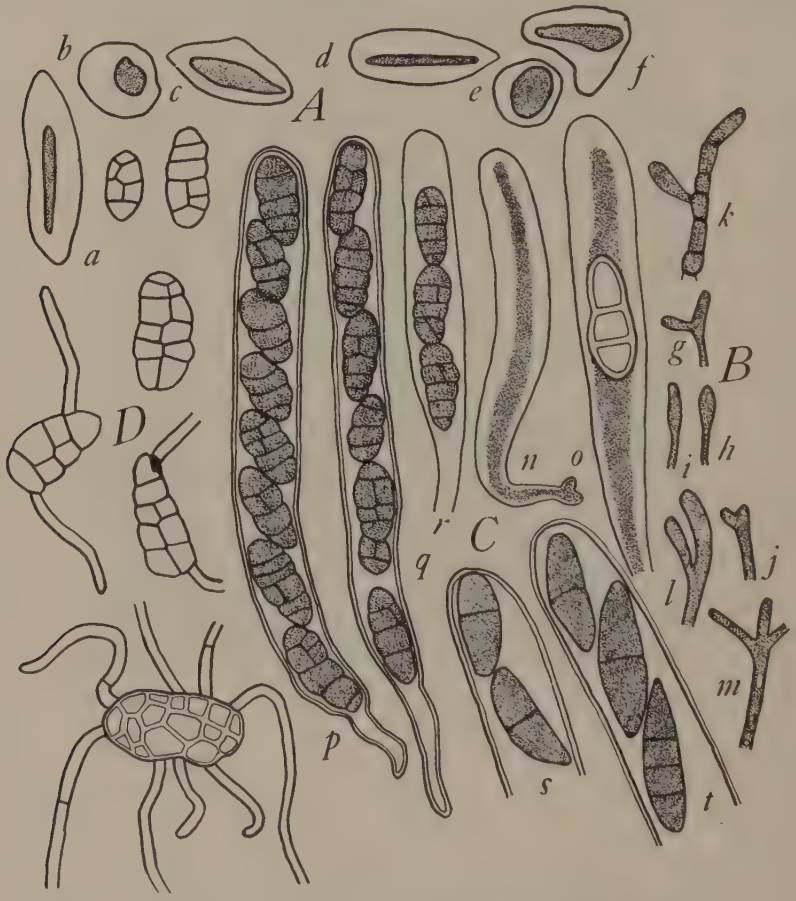

FIG. 2. A. Forms of perithecia as seen from above: navicular, a, d; hysterothecial type, a, c, d; angular, f; hemispherical, b, e. × 12. B. Tips of epithecial hyphae: branched, g, j–m; simple, h, i. g–j, × 600; k–m, × 1240. C. Forms of asci and ascospores: n, immature; o, r, aberrant forms; p, q, predominant forms; s, t, tips of asci with atypical ascospores. n–r, × 600; s, t, × 1240. D. Septation and germination of ascospores, × 600.

brown. The greater numbers have blunt, rounded ends, but occasional spores are somewhat acute (FIG. 2C, s, t). The longest of 20 measured was 33.5 μ, the shortest 17 μ, the average 27 $\times$ 13 μ.

The red periphyses, interwoven to form the epithecium, appear to be distinctive in color: species of *Hysterographium,* described in the Sylloge Fungorum (2), Kryptogamenflora (1), and other taxonomic literature available to the writer, have yellow or brown epithecia. Tips of the periphysate hyphae may be simple (FIG. 2B, h, i) or branched (FIG. 2B, j–m), swollen or uniformly cylindrical.

This species of *Hysterographium* has not been seen by the author on any substrate other than the skeletal framework of the cholla cactus, but no special survey has been made for it.

Hysterographium opuntiae sp. nov.

Peritheciis dense gregariis in serie parallela vel paucigregariis vel sparsis, nigris, erumpenti-superficialibus, sessilibus, non striatis, regulariter naviculariformibus rima longitudinali dehiscentia (hysterotheciis) vel angulatis vel hemisphaericis apice rotundatis, 1.7–0.64 mm medio longis, 5 mm in longitudine extremis, 0.50 mm in longitudine minimis; ascis clavatis vel elongato-clavatis, apice rotundatis, basi angustatis, rectis vel curvatis, hyalinis, brevissime stipitatis, plerumque 3–8-sporis, 85–160 μ longis $\times$ 12.5–16 μ latis; sporidiis oblique monostichis, forma et magnitudine variis, rectis vel leviter curvulis, obovatis vel oblongo-ellipsoideis vel naviculariformibus, 3–5-septatis (accidentibus septis longitudinalibus 1–3), ad septa non vel insigniter constrictis, 17–33 μ longis $\times$ 13 μ medio latis, brunneis; paraphysibus simplicis vel ramulosis, apice rubidis.

Habitat in ligno decorticato *Opuntiae fulgidae* in Arizona meridionali.

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THE CURTIS COLLECTION AT BROWN UNIVERSITY

Walter H. Snell and Esther A. Dick

From the earliest days of any teaching of botany in Brown University,[1] other than a course in medical mycology in 1897–98, there was no particular interest in mycology until the period 1910–1920, in the early part of which a branch of the federal Office of Forest Pathology was established in collaboration with the Department of Botany and in the latter part of which C. W. Dodge and the senior author of this note arrived here. The older botanists were taxonomists chiefly in the higher groups of plants, but on the other hand were interested in all plant organisms. This was especially true of W. Whitman Bailey, who was the Department of Botany, with an occasional assistant, from 1877 to 1906. He not only started the present herbarium with his own but he began to build up a botanical library and to enlarge the herbarium with the very generous assistance in both directions of Shephen T. Olney, a Providence industrialist. As to the library, President Benjamin Andrews said in 1890—"In no department, except Botany, are we able to have all the latest critical works"—and in 1894, "The strongest department of the Library is Botany and in this the most complete collection is on Floras." Those of us who have come later have been fortunate in having available many of the important mycological works of the last century or before, such as those of Bulliard, Fries, Persoon, Corda, Saccardo, etc., and several inclusive floras.

Similarly, although the phanerogamic herbarium is chiefly local in view of the proximity of the imposing and extensive herbaria

[1] Lectures on "Natural History in General, Mineralogy and Botany in Particular" given at Rhode Island College (now Brown University) in 1786 and 1787, "First of the kind to be given in the New World," page 3, in Courtney, J. W. Benjamin Waterhouse, M.D. Communication faite au Vme. Congrès International d'Histoire de la Médecine. Geneva. 1926.

of Gray and of the New England Botanical Club, Bailey gathered a few collections of fungi that were available. A master stroke was the acquisition of a set of the M. A. Curtis collections.

Curtis's main herbarium was purchased after his death by Farlow and is now in the Farlow Herbarium in Harvard. In a letter to Howe dated March 22, 1870, about two years before his death,[2] Curtis expressed a desire to dispose of his "spare Fungi," guaranteeing 1500 species and perhaps 2000 or more (p. 199). Shear and Stevens (*l.c.*, p. 200) state that they do not know how many sets were prepared but that "one appears to have been purchased by Peck and another by Bessey, whose herbaria are respectively in the New York State Museum at Albany and in the herbarium of the University of Nebraska at Lincoln."

It does not seem to be very widely known that still another set is in the herbarium at Brown University, acquired by Bailey in 1872 as far as can be learned, although possibly earlier.

Since the date of acquisition, the Curtis specimens here have lain practically undisturbed, except for an occasional inspection or loan to someone who learned of our possession. The specimens were glued on pieces of paper, with Curtis's labels attached. The herbarium sheets were in miscellaneous folders, scattered amongst other collections. It has long since been high time that better care was given to the collection, not only with regard to availability and as a matter of protection to these valuable items but also because the ink of Curtis's hand has begun to fade. It has been the intention of the writers to do something about this matter for at least 25 years, but various preoccupations, serious or trivial, have interfered.

During the past fall, however, time and personnel were available, and the work was completed, chiefly through the efforts of Miss Jacqueline Vestal, a graduate student in the Department, and the junior author. The specimens with the original labels have been packeted, and the labels have been copied verbatim in India ink, with the inclusion of such annotations as were available. Miss Vestal did an excellent piece of work in deciphering some of

[2] C. L. Shear and Neil E. Stevens. The mycological work of Moses Ashley Curtis. Mycologia **11**: 181–201. 1919.

the labels on which the ink had faded or disappeared in varying degrees, in some cases completely and with only the impressions of the pen visible, and the writers collaborated in searching the literature for lost words or letters. The specimens have been arranged alphabetically, as named by Berkeley, in herbarium folders also properly labelled for easy finding of any specimen, in one section of our herbarium instead of scattered amongst other exsiccati.

It is not known to the present writers how many specimens are contained in the main Curtis Herbarium at Cambridge or in the sets purchased by Peck and Bessey, but there are in our set 1627 packets, with at least four packets each containing leaves or twigs bearing two species.

It has been the view of these writers that this information might be at least of interest and probably of value to American and Canadian mycologists.

BROWN UNIVERSITY,
PROVIDENCE, RHODE ISLAND

NOTES AND BRIEF ARTICLES

Pure culture syntheses of pine mycorrhizae in Terra-Lite

In 1921–1922, Melin [1, 2] successfully demonstrated for the first times that ectotrophic mycorrhizae could be produced in synthetic cultures by inoculating seedlings of *Picea abies, Pinus silvestris* and *Larix europaea* with appropriate fungi. Thereafter, he and other investigators have applied this basic technique for studies in determining the identity of mycorrhizal fungi and in physiological experiments dealing with mycorrhizae. The most used and most successful substrate for synthesis cultures has been sand moistened with a nutrient solution. This combination has been especially adaptable in experiments requiring sterile conditions and definite knowledge concerning the composition of the substrate.

Although sand has been proven suitable for such experiments, it has some properties which render difficult its use under certain conditions, especially in pure culture experiments. It must be chemically cleaned for removal of undesirable inorganic and organic substances, has a relatively small surface area and a low water holding capacity. Since evaporation of water from this substrate is rapid at ordinary greenhouse temperatures, frequent liquid replacement is necessary in aseptic synthesis cultures and contamination risks are great.

In an attempt to find a medium which would overcome the disadvantages of sand, the author began experiments at the George Washington University early in 1952 using Terra-Lite, a product of Vermiculite Products Inc., Houston, Texas. The method first developed was further refined in studies at the Institute of Physiological Botany, Uppsala, Sweden in experiments involving ectotrophic mycorrhizae.

[1] Melin, E. Über die Mykorrhizenpilze von *Pinus silvestris* L. und *Picea abies* (L.) Karst. Svensk. Bot. Tidskr. **15**: 192–203. 1921.

[2] Melin, E. *Boletus*-Arten als Mykorrhizenpilze der Waldbäume. Ber. Deutsch. Bot. Ges. **40**: 94–97. 1922.

For experiments in the United States, Tella-Lite from the source mentioned above was used, while for use in Sweden it has been obtained through C. Gartenmann and Son, S.A., Berne, Switzerland. According to George E. Ziegler [3] (Personal Communication), the following are some of the more important characteristics of Terra-Lite: It is biologically sterile and void of organic material since it is expanded at 1800° F and sealed in bags at a temperature in excess of 200° F. A chemical analysis reveals no constituents unfavorable to growth of higher plants or to fungi. It has good base exchange characteristics, the exchange capacity ranging from approximately 10 to 18 milli-equivalents per hundred grams, the predominating exchangeable base being calcium. The buffering capacity is only slightly greater than that of an inert material such as sand.

The author has found that if Terra-Lite is screened to remove the fine dust-like particles, then saturated with the nutrient solution prescribed for pure culture syntheses of mycorrhizae by Melin [4] as modified by Norkrans,[5] and immediately thereafter autoclaved, it increases by about one-third in volume due to further expansion of the particles. It retains about six times the amount of solution per gram dry weight, and provides excellent conditions for aeration. The solution should be adjusted to pH 4 with an acid such as HCl prior to autoclaving, resulting in a final pH of 5.2–5.4. Terra-Lite evidently possess properties which tie up hydrogen ions during the process. To have uniform conditions of depth of the substrate, measurements should be done by volume rather than dry weight alone since the variation in particle sizes may produce different volumes of substrate among the cultures. Depending upon the amount of substrate and the amount of excess solution in the cultures, it is possible to grow pines aseptically for a short time up to several months without having to

[3] Director of Research of the Zonolite Company, Chicago, Illinois.

[4] Melin, E. Methoden der experimentellen Untersuchung mykotropher Pflanzen. Abderhalden: Handbuch d. biol. Arbeitsmeth. Abt. XI, **4**: 1015–1108. 1936.

[5] Norkrans, B. Some mycorrhiza-forming *Tricholoma* species. Svensk. Bot. Tidskr. **43**: 185–490. 1949.

supplement liquid. If permitted to dry, the particles tend to return to their original volume and increase in size only slightly if solution is thereafter added.

In April, 1952, *Pinus virginiana* seeds were sterilized and germinated as described by Hacskaylo.[6] Two liter Erlenmeyer flasks were filled to a depth of 5 cm with Terra-Lite and to this was added 720 ml nutrient solution (see above). The flasks were then autoclaved at 15 lbs pressure for 20 minutes. After the medium had cooled, seedlings with radicles approximately 15 mm in

FIG. 1. Mycorrhizae of *Pinus virginiana* growing in pure culture. ×6.

length were planted in the centers of the flasks. A long inoculating needle was used to transfer the seedlings from petri dishes into the flasks, and for the planting. At the same time, the flasks were inoculated with mycelium of *Lepiota rhacodes*. The cultures were then placed in a greenhouse and no solution was added thereafter.

Four months later, a study of the cultures showed extensive

[6] Hacskaylo, E. A study of the roots of *Pinus virginiana* in relation to certain hymenomycetes suspected of being mycorrhizal. Jour. Wash. Acad. Sci. **41**: 399–400. 1951.

root development and heavy mycelial growth in the medium. The shoots had begun to develop lateral branches, were an average of 15 cm tall and had green, healthy appearing leaves. Further

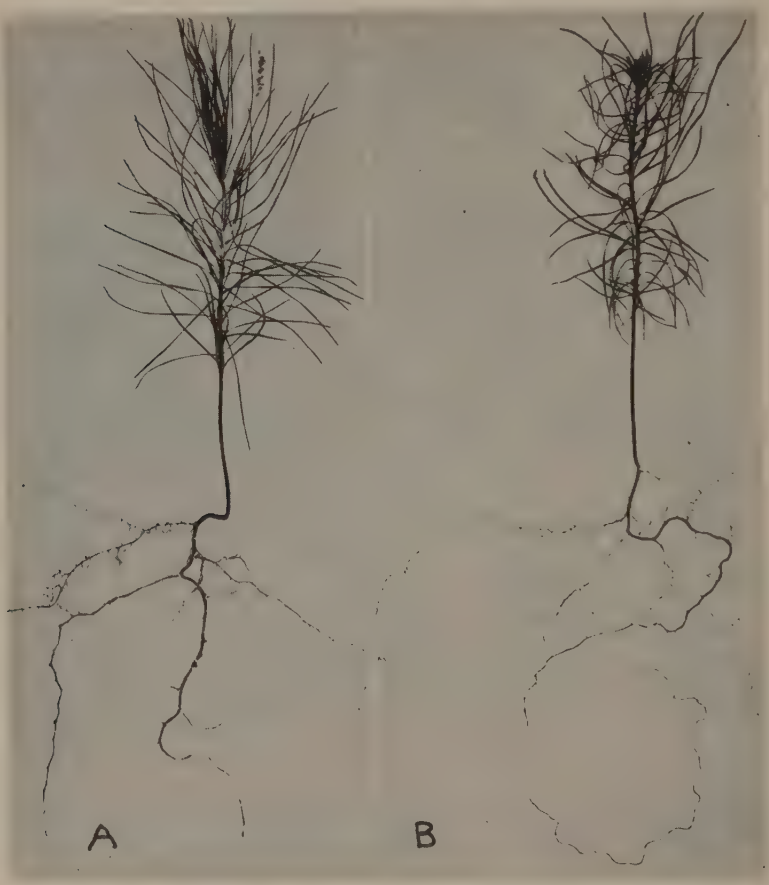

FIG. 2. Nonmycorrhizal plants of A. *Pinus virginiana* and B. *Pinus silvestris* grown in Terra-Lite. 3 and 4 months old respectively ½ natural size.

examination of the roots revealed well developed ectotrophic mycorrhizae of the unbranched and singly forked types (FIG. 1).

In Sweden, the author has grown *Pinus silvestris* in 500 ml culture flasks using a combination of approximately 15 grams

Terra-Lite and 120 ml nutrient solution. After having grown for two months the seedlings were inoculated with suspensions of *Boletus variegatus* and *Rhizopogon roseolus,* known to be mycorrhizal on the tree species,[7, 8] according to a procedure used by Melin (Unpublished). At the same time 20 ml nutrient solution was added to each culture flask. After one additional month, the seedlings had developed shoots from 8 to 12 cm in height, were actively growing and had healthy-appearing foliage. Root growth was extensive and the fungi had spread densely throughout the medium. Ectotrophic mycorrhizae of the unbranched, singly forked and coralloid types were abundant in cultures with both fungi.

Under the conditions of these experiments wherein Terra-Lite moistened with a nutrient solution was used as a substrate, it was evident that the medium serves well for pure culture syntheses of ectotrophic mycorrhizae. The advantages in using this medium rather than sand are: It needs no previous treatment before use It has a high water holding capacity and eliminates frequent additions of liquid, thereby reducing contamination possibilities. It provides excellent aeration conditions for pine roots and fungi and their rapid overall development resulting in mycorrhizal formation may reduce the time needed for well-developed mycorrhizal plants during experiments. This technique is being applied in further investigations concerned with the physiology of the mycorrhizal phenomenon.

The author is grateful to Professor Elias Melin of the Institute of Physiological Botany, Uppsala, for providing the facilities for the portion of this work done in Sweden, to the American Scandinavian Foundation for funds to study in Sweden, and to Dr. Marvin I. Gottlieb and Å. Danielsson for assistance in preparation of the photographs.—EDWARD HACSKAYLO, Department of Botany, The George Washington University, Washington, D. C.

[7] Melin, E. Experimentelle Untersuchungen über die Konstitution und Ökologie der Mykorrhizen von *Pinus silvestris* und *Picea abies.* Mykol. Unters. u. Ber. von R. Falck. **2**: 73–331. 1923.

[8] Modess, O. Zur Kenntnis der Mykorrhizabildner von Kiefer und Fichte. Symb. Bot. Ups. V: 1. pp. 1–146. 1941.

SYNCHYTRIUM CHAMAEDRYOIDIS

In a previous paper the author [1] called attention to Cook's [2] *S. urticae* and noted that this binomial was used by Sorokin [3] in 1873. Since the publication of this note the writer has found an abundance of Cook's fungus on *Urtica chamaedryoides* near Austin, Texas. During a study of this species in the living condition the bodies which Sorokin believed to be the resting spores of a *Synchytrium* species also were found in great abundance. The latter were present in healthy as well as dying leaves of *H. chamaedryoides*, and an examination of several other species of *Urtica* showed that fundamentally similar but slightly different bodies were present and fairly evenly distributed in all leaves.

A careful study of their development from early to mature stages has revealed that they are the stalked cystoliths or crystals which occur commonly in the Urticaceae, as shown by Wedell [4] Chareyre,[5] Werner,[6] Freisleben,[7] and others. In *U. chamaedryoides* the small cystoliths may be subspherical and 20–31 μ in diameter. The majority, however, are oval to oblong, bean-shaped or slightly reniform, 13–67 × 19–221 μ. In the young stages of development the lithocyst or mother cell is only slightly larger than the neighboring cells and only partly filled by the cystolith. However, at maturity the lithocyst may be twice the size of adjacent cells and almost completely filled by the crystal.

The pore which Sorokin described apparently is the stalk in cross section, and when viewed directly from above it may appear

[1] Karling, J. S. 1953. *Synchytrium urticae*. Mycologia **45**: 613–615.

[2] Cook, M. T. 1952. Species of *Synchytrium* in Louisiana. VIII. A new species on *Urtica chamaedryoides*. Mycologia **44**: 827–828.

[3] Sorokin, N. 1873. A few words about a new fungus, *Synchytrium urticae*. Contrib. 3rd Meeting Russian Sci. Kiev, Bot. Transact. pp. 39–42, pl. 3 (Trans. from Russian).

[4] Wedell, A. 1854. Sur les cystolithes ou concretions calcaires des Urticees et d' autres vègètaux. Ann. Sci. Nat. Bot. ser. 4 t. II: 267.

[5] Chareyre, J. 1884. Nouvelles recherches sur les cystoliths. Rev. Sci. Nat. 3 ser. **3**: 523–602; **4**: 1–105.

[6] Werner, O. 1931. Haar- und cystolithenscheiben in Blatt geweben bei Urticales, bei *Bryonia dioica* und *Zexenia longipetiolata*. Oesterr. Bot. Zeitschr. **80**: 81.

[7] Freisleben, R. 1933. Untersuchungen über Bildung and Auflösung von Cystolithen bei den Urticales. Flora **127**: 1–45.

as a depression surrounded by an almost circular, clear zone. The surface of the cystolith is very uneven or densely verrucose, and as a whole it appears greyish-white to gleaming in appearance. It is also relatively fragile and may be crushed readily by pressure on the cover glass. In all respects it appears to be identical with the resting bodies described by Sorokin.

Since publication of the previous note the author has reexamined, through the courtesy of the New York Botanical Garden, the original herbarium material collected by Sorokin on *U. dioica* and distributed by C. Roumeguere as Fungi selecti exsiccati No. *1737*. In this material the cystoliths are fairly evenly distributed and stand up as oval or oblong, greyish-white bodies on the surface of the leaves. Apparently, their slightly raised appearance is due to the drying and collapse of the adjacent cells.

The gall-like areas which the author reported from the same material previously have been reexamined critically also. These areas or spots are oval, subspherical, and elongate with a slightly raised border and are almost hollow except for the lower epidermis of the leaf. They do not resemble *Synchytrium* galls, and it is very doubtful that they were caused by a species of this genus. A few mycelial fragments and conidia of another fungus are present in a few of them, and this organism may have caused the spots or entered as a secondary invader. Therefore, it seems very doubtful that more than one species of *Synchytrium* has been reported on *Urtica*. In this connection it may be noted that all attempts to infect *U. dioica* with Cook's species have failed so far.

Cook's *S. urticae*, on the other hand, is a true species of *Synchytrium*, and the author has confirmed his observations from the material collected in Texas. Infected *U. chamaedryoides* plants are growing in the greenhouse at Purdue University, and from these it has been possible to work out the life cycle and type of development of this species. However, inasmuch as the binomial *S. urticae* has been preempted by Sorokin's illegitimate or erroneous use of it, it is not valid for Cook's species, according to Art. 74 of the rules of nomenclature. Accordingly, the name **S. chamaedryoidis** nom. nov. is proposed for his species.—JOHN S. KARLING, Purdue University.

Syn. n. *S. urticae* M. T. Cook,

CORRECTION FOR THE SUBGENERIC NAME EUCAMPTOMERIS

Dr. D. P. Rogers has called to my attention the recent ruling of the International Botanical Congress at Stockholm, in Article 32 of which it is directed that the name of a subgenus in which the type of the genus is included should be the same as the genus name. Therefore the subgeneric name *Eucamptomeris* (Mycologia **45**: 364–390. 1953) should be changed to *Camptomeris*.—ERNST A. BESSEY, Michigan State College.

SAKSENAEA VASIFORMIS—A CORRECTION

In the paper by Saksena: A new genus of the Mucorales (Mycologia **45**: 426–436. 1953) the magnifications given apply to the original figures, not to the reductions as published. Figs. 1–21, p. 427 and 22–30, p. 429, are reduced to ⅓ of the original size and Figs. 39–50, p. 432, to ¾ of the original size. The magnifications given are to be correspondingly reduced.

ANNOUNCEMENTS CONCERNING APPLICATION FOR GRANTS IN 1954

A portion of the annual income received from Sustaining Memberships is set aside each year for grants-in-aid to regular members of the Mycological Society of America. These grants are awarded "to promote interest" and "to further research" in the field of mycology, and they ordinarily do not exceed $100 each. Application blanks may be obtained from Dr. C. J. Alexopoulos, Secretary-Treasurer, Department of Botany, Michigan State College, East Lansing, Michigan. Applications, submitted in triplicate, must be in the hands of the Chairman of the Research Grants Committee by February 1, 1954.

Applications are not evaluated on any set of rigidly fixed standards, and the Committee will welcome a wide variety of requests. The funds available are not large, however, and it seems appropriate at this time to indicate the general philosophy which has been developed as a guide to making awards. The Committee feels that small grants will promote interest and research in mycology most effectively if they are awarded for the most part where the needs are most acute. Special consideration will be given, therefore, to those applicants who have least access to other research funds: to younger mycologists in the early years of their careers, to investigators in smaller institutions where internal funds are particularly limited, and to those who are engaged upon projects which do not ordinarily derive ready support from large granting agencies such as the Institutes and Foundations. Younger applicants are urged to provide supporting recommendation from some senior research investigator with whom they have been associated. Applications for funds to defray expenses of collecting trips will be considered only when they reveal urgent need closely associated with a sound

research program. With the limited funds at its disposal, the Committee can not, unfortunately, consider applications for support of graduate student research.

Members of the Committee on Research Grants:

JOHN N. COUCH—University of North Carolina, Chapel Hill, North Carolina.

JOHN EHRLICH—Research Laboratories, Parke Davis Co., Detroit 32, Michigan.

RALPH EMERSON (Chairman of the Committee)—Department of Botany, University of California, Berkeley 4, California.

REPORT OF THE MANAGING EDITOR OF MYCOLOGIA

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR THE YEAR ENDED JUNE 30, 1953

Cash receipts		
Joint funds		
Members' subscriptions (Mycological Society)	$ 2,692.00	
Other subscriptions	4,421.46	
Sales of V. 25 and later	282.40	
Excess pages and illustrations	496.38	
Reprints	11.25	
	$ 7,903.49	
Special funds		
Sales of V. 1–24 and Index	61.00	
Income from endowment	705.00	
	$ 766.00	
Total receipts		$ 8,669.49
Cash disbursements		
Printing and distribution	$10,059.49	
Reprinting	579.31	
Office expense	407.85	
Total expenditures		$11,046.65
Excess of expenditures over receipts		2,377.16
Unexpended balance July 1, 1952		6,001.94
Unexpended balance July 1, 1953		$ 3,624.78
Endowment		$14,000.00

The above MYCOLOGIA funds are administered by the New York Botanical Garden, and the balances at June 30, 1953, are in agreement with the amounts shown in the financial statements of that organization which have been examined by Price, Waterhouse & Company.

DONALD P. ROGERS, *Managing Editor*

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